UREA NITROGEN UTILIZATION AS AFFECTED BY DIETARY MINERAL SALTS AND MANIPULATION OF RUMINAL KINETICS

BY

MANUEL HUMBERTO RUILOBA

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Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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By

Manuel Humberto Ruiloba

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Objectives were to study in vitro and in vivo effects of macromineral salts on dietary urea-nitrogen utilization in ruminants. In two in vitro factorial experiments, effects of potassium (KCl) on microbial urea utilization were studied. Added potassium (K) and urea (U) concentrations varied between 0 to 4.53 g and 0 to 429 mg/liter, respectively. Microbial response was evaluated as apparent N utilization (AMNU), initial urea-N and NH_3-N (from ruminal fluid) content minus final NH_3-N content. Results were quite similar in both experiments. Day of ruminal fluid collection modified effect of K, U and T. There were interactions ($P<.001$) among K, U and fermentation interval (T). Added K decreased AMNU only slightly between 0 and 1.50 g K/liter. Urea and T increased AMNU.

Lactating Holstein cows fitted with ruminal and abomasal cannulae were used in a simple reversal design to study the effect of changing ruminal kinetics with dietary NaCl and KCl on extent and efficiency of utilization by ruminal microorganisms. Dietary content of sodium and potassium were .19 and 1.03, and .58 and 1.86 percent (dry basis) for control (CT) and high-mineral treatment (HMT). Dietary urea content was 1.5 percent. Ytterbium, CrEDTA and lignin were used as ruminal kinetic markers and cytosine and adenine as microbial markers. High-mineral treatment increased water intake ($P<.001$) and ruminal volume ($P<.01$) but did not affect dilution rate and solid turnover rate ($P>.10$). Ruminal digesta outflow was increased from 170.5 to 242.8 kg/day ($P<.01$) by HMT. Ruminal microbial N outflow ($P<.01$) and efficiency ($P<.001$) were increased by HMT. Both estimates were not different ($P>.10$) as estimated by either microbial marker. Average microbial N outflows were 246.4 and 314.6 g/day and efficiencies were 18.6 and 29.4 g/kg OM truly fermented in rumen with CT and HMT, respectively. High-mineral treatment decreased OM disappearing in rumen ($P<.001$) but did not affect apparent OM and ADF digestibility, N balance and lactational performance ($P>.10$).

Study indicated a negative effect of added KCL on in vitro microbial urea utilization and a positive effect of increased ruminal kinetics resulting from dietary mineral salts on urea utilization by ruminal microorganisms.

CHAPTER 1

INTRODUCTION

A century ago it was known ruminants have a capacity for utilizing non-protein nitrogen (NPN). Since then, many researchers have studied ways of increasing utilization of NPN, especially urea, which is one of the most available sources of nitrogen (N) for ruminants. This is due to the facts that, as a source of crude protein, urea and other forms of NPN are cheaper than natural protein, and animal production increasingly competes with humans for the same natural proteins. Physical, chemical and biological methods to improve utilization of urea by ruminants have been studied but most have practical and economical limitations.

In recent years, many researchers have studied the effects of changing ruminal kinetics on various digestive parameters and methods of manipulating it to improve ruminal fermentation efficiency. Different studies have pointed out a positive effect of dilution rate (fraction of ruminal liquid volume replaced by hour) on efficiency of microbial synthesis; this suggests that an increase in ruminal turnover rate or ruminal flow may improve utilization of NPN because ruminal capacity to convert nitrogen to microbial protein would be improved. This could have considerable

practical implications since estimations by Roy et al., cited by Smith (1979), indicate that with a microbial yield corresponding to a Y(ATP) of 14.5, a high-producing dairy cow could make use of dietary NPN only if the diet contained protein of exceptionally low degradability. If a Y(ATP) of 20 is assumed, that animal could derive virtually all its protein requirements from dietary NPN. However, there is little experimental evidence verifying these estimations.

Different methods have been used to change ruminal dilution rate and ruminal flow. Alteration of ruminal osmolality with sodium salts has been one of the most effective. Potassium salts may be another alternative since potassium is required by ruminants at a relatively high dietary concentration, and ruminants can tolerate high quantities, in excess of requirements.

Recent results from Florida (Beede et al., 1983a, 1983b) have indicated higher sodium and potassium requirements for lactating cows, especially those under heat stress, than recommended by the NRC (1978). Although different experiments have been conducted in Florida to study nutritional and physiological effects of these new levels of sodium and potassium on performance of lactating cows, no information is available with respect to their effects on ruminal dynamics and microbial protein synthesis. Therefore, in vitro and in vivo studies reported herein were undertaken with general objectives:

(1) to assess effects of varying potassium (KCl) concentrations on apparent microbial nitrogen utilization in vitro;

(2) to determine effects of sodium and potassium chloride on ruminal kinetics;

(3) to determine effects of sodium and potassium chloride on urea utilization by ruminal microorganisms;

(4) to determine effects of ruminal kinetics on urea utilization by ruminal microorganisms.

CHAPTER 2

LITERATURE REVIEW

Ruminal Turnover Rate

Turnover time is defined as time required for equivalent of one volume of culture or digesta to be replaced, whereas turnover rate is the fraction of culture or digesta volume replaced per hour (Isaacson et al., 1975). Turnover time is the inverse value of turnover rate (1/turnover rate equals turnover time). Usually turnover rate is expressed as percent per hour. The terms dilution rate (D) and solid retention time (SRT) are used commonly for the liquid and solid or particulate phases of digesta, respectively.

In evaluating the literature dealing with ruminal turnover and related aspects, it is important that liquid and solid turnover be considered independently because they do not necessarily occur at the same rate (Bull et al., 1979). For instance, turnover rate of bacteria generally is measured by dilution rate and not by the absolute turnover rate of bacteria. Nevertheless, bacteria can be associated with particles which would cause their turnover to lag behind the fluid turnover rate by 50 to 300 percent (Owens and Isaacson, 1977).

Turnover rates of liquid and solid phases or physical and chemical constituents of digesta depend on dietary and physiological factors affecting ruminal kinetics. For example, Owens and Isaacson (1977) indicated that, as fiber was added to the diet, turnover rate of liquid, bacteria, and small particles increased while that of large particles tended to decline. Particle size and specific gravity are two primary factors controlling potential exit of feed particles from the reticulo-rumen. Therefore, ruminal outflow does not affect ruminal disappearance of these materials until the proper particle size is reached (Bergen and Yokoyama, 1977).

A wide range of values has been reported for both dilution rate and solid retention time in ruminants. Crawford et al. (1980b), in a review of literature, reported dilution rates as low as 2 percent/h for fasting cows and as high as 33 percent/h for mid-lactation cows. They also indicated solid retention times ranging from 5 to 50 h.

Most studies have shown a narrower range of approximately 5.5 to 15.5 percent/h for dilution rate and 10 to 35 h for solid retention time. Ruminal dilution rate averaged 7.3 for sheep (n=33) and 7.5 percent/h for cattle (n=23) (Evans, 1981a). Solid retention time averaged 20.1 h for sheep (n=35) and 25.6 h for cattle (n=61) (Evans, 1981a).

There are a number of factors, including dietary components and characteristics and rates of feeding, which

may affect liquid and (or) solid turnovers. Each of these factors may influence ruminal conditions, microbial species and end-products of fermentation (Bull et al., 1979). Increased dilution rate affects ruminal microbes and their activities, ruminal degradation of feed constituents, and the metabolic and nutritional status of the animal (Chalupa, 1977). This may be important in terms of improved microbial growth efficiency, increased utilization of nonprotein nitrogen, and reduction of fermentation losses for feeds that can be digested in the intestine. Several of these factors are discussed subsequently.

Effect of Turnover Rate on Ruminal Microorganisms

Importance of dilution rate on in vitro ruminal bacterial growth was described by Hobson (1965), who found that microbial growth efficiency, Y(ATP) (microbial yield, dry weight, per mole of ATP produced in the rumen), could be altered by varying the dilution rate. This information has been corroborated in vivo with different ruminant species and diets (Cole et al., 1976; Kennedy et al., 1976; Prigge et al., 1978). In addition, dilution rate may change microbial cell composition (Isaacson et al., 1975; Bergen and Yokoyama, 1977; Chalupa, 1977). Bergen and Yokoyama (1977) indicated that serious attempts to manipulate dilution rate should take into account the following factors: (1) decreased feed digestibility, (2) decrease in ruminal protozoa numbers, (3) possible changes in

macromolecular composition of microbial cells, and (4) possible changes in fermentation end-products. Crawford et al. (1980a, b) and Hoover et al. (1982) reported results from in vitro studies indicating the importance of solid retention time on microbial activity and possible interactions between solid retention time and dilution rate.

Dilution rate affects the species of bacteria (Hungate, 1966) and protozoa (Coleman, 1975) present. Latham and Sharpe (1975) studied the effects of dilution rate on ruminal microbial species in lambs fed a diet based on flaked corn and pelleted dry grass. Selenomonads and bacteroides were predominant in animals with low dilution rates but bacteroides decreased and gram-variable bacteria, mainly chain forming cocci, predominated when dilution rates were increased by dietary infusion of inorganic salts. Numbers of cellulolytic bacteria varied widely between animals with all diets but there were no consistent differences among diets for counts of butyrivibrio or protozoa. In an experiment using dietary salts to change dilution rate in sheep, Thomson et al. (1978) found that selenomonads and bacteroides were predominant at low dilution rates, but at higher dilution rates, bacteroides were replaced by large numbers of chain-forming cocci. Protozoa numbers varied among treatments.

Abe and Kumeno (1973) studied effects of dilution rate on protozoa numbers using an in vitro continuous culture

system. They found that at dilution rates greater than 1.0 fermenter volume per day, protozoa numbers declined because the generation time required by many species was greater than residence time in the fermenter and a washout of protozoa occurred. Results suggested that dilution rates lower than 1.0 fermenter volume per day may cause a decrease in protozoa number due to an increase in concentration of fermentation end-products and a decrease in pH of the culture. Results obtained at high dilution rates in this experiment did not agree with in vivo results reported by Hungate (1966) for animals with dilution rates exceeding 1.5 volumes per day. A possible explanation is that protozoa removal from the rumen occurs at a slower rate than fluid removal. Weller and Pilgrim (1974) confirmed this presumption when they found that the passage of protozoa to the abomasum was only 6 to 9 percent of that predicted from fluid rates. It would appear that protozoa undergo some sort of sequestration in the rumen.

Effects of dilution rate on protozoa numbers may not be a serious problem because it seems that the contribution of ruminal protozoa to net microbial protein passage is not large (Bergen and Yokoyama, 1977). But this may depend on fiber content of the diet and productive function of the animal. Of considerable interest may be effect of increased dilution rates on changes in the chemical composition of

microbial cells produced in the rumen. Nevertheless, there is little information regarding this aspect.

In general, the main nitrogenous compounds of ruminal bacteria are proteins and nucleic acids which contain about 75 to 85 and 13 to 19 percent of total nitrogen, respectively (Smith, 1979). Nitrogen content of protozoa is low compared to bacteria because of higher polysaccharide content (Hungate, 1966). Nitrogen contents of bacteria and protozoa are approximately 8.8 and 6.1 percent (dry basis), respectively (Chalupa, 1972).

Kjeldegaard, cited by Bergen and Yokoyama (1977), indicated that as dilution rate increases, relative content of DNA and protein in ruminal microorganisms declines. In vitro study of Bates, cited by Bergen et al. (1982), with different bacterial species, indicated that the RNA-to-protein ratio increased at a rate of .68 as the specific growth rate increased. Herbert (1974) indicated that as the exponential growth rate increases, microbial RNA content increases but DNA and protein decline slightly.

Isaacson et al. (1975) found that carbon and nitrogen content of cells increased when dilution rate increased from 2 to 12 percent/h. They indicated that, with an accumulation of carbohydrates in cells, lowered nitrogen content would be expected; however, the opposite was observed. Authors suggested that these results may be due to a change in type of bacterial population or an effect

of the faster-growing bacteria. The latter possibility was suggested based on data of Maale, cited by Isaacson et al. (1975), who found that at medium and high growth rates, the number of ribosomes was proportional to the growth rate. The latter point needs more clarification because if an improvement in cell yield is at the expense of microbial protein quality and quantity, as a result of an increase in nucleic acid yield, it will be an obvious disadvantage to the animal.

Maintenance requirements of bacteria, as in other biological systems, must be met in preference to growth, and any excess energy is then available for microbial growth. Maintenance requirement is not a constant. Factors such as dilution rate can affect it. Stouthamer and Bettenhaussen, cited by Bergen and Yokoyama (1977), studying the Y(ATP) yield for Aerobacter aerogenes, grown at various dilution rates in an energy-limiting chemostat, were able to show that slow-growing organisms, which were associated with low dilution rates, have a much higher energy maintenance coefficient. Another effect of dilution rate on microbial maintenance requirements was demonstrated by Isaacson et al. (1975). Using mixed ruminal cultures grown at various dilution rates in a continuous culture system with glucose, they found that at the lowest dilution rate (2 percent/h) approximately 55 percent of the energy derived from glucose was used for maintenance; at the highest dilution rate (12

percent/h), only 15 percent of the energy was used for maintenance.

To estimate the cell requirement, Isaacson et al. (1975) used the Pirt's method, which consists of plotting the reciprocal of Y (glucose) (cell yield per mole glucose fermented in the rumen) or Y(ATP) against the reciprocal of the dilution rate; the resulting slope is the maintenance coefficient (moles of substrate per g-cell per h). Using the same method to estimate maintenance, Harrison and McAllan (1980) found that, at a dilution rate of 2 percent/h, 65 percent of the energy used by a bacterium was for maintenance; this decreased to 32 percent at a dilution rate of 6 percent/h. These data illustrate the marked sensitivity of microbial yield to alterations in dilution rates when the operating dilution rate is very low. Importance of maintenance requirements on Y(ATP) also has been indicated by others (Isaacson et al., 1975; Bergen and Yokoyama, 1977; Hespell, 1979).

Several authors (Harrison et al., 1975; Isaacson et al., 1975; Kennedy and Milligan, 1978) have reported a positive correlation between microbial yield or microbial efficiency of growth and dilution rate. Walker et al. (1975), who fed sheep diets based on dried and fresh forages, obtained a significant increase in microbial synthesis per 100 g of OM digested in the rumen when microbial turnover rate was increased.

Isaacson et al. (1975), using a rumen fluid medium in a chemostat at three dilution rates (2, 6 and 12 percent/h), noted a Michaelis-Menton-type saturation curve when Y(ATP) was plotted against dilution rate. This response indicated that with increasing growth rates, a greater proportion of the energy derived from the substrate was used for net culture growth. In this experiment, number of viable cells per mole of glucose fermented increased and the amount of ATP produced per mole of glucose fermented decreased as dilution rate increased. Increased dilution rate from 2 to 6 percent/h improved cell yield but there was no improvement from 6 to 12 percent/h. Authors pointed out that a faster dilution rate would increase microbial cell or protein production in two ways: (1) with a constant concentration of cells and rumen volume at each dilution rate, an increase in dilution rate at the same energy concentration in the diluting medium will increase output of cells; and (2) since efficiency of utilization of energy by bacterial cells increases with increasing dilution rates, cell concentration will be higher at higher dilution rates and total yield will be enhanced. The same authors found that as dilution rate increased, cell concentration increased as well. However, others have found opposite results (Thomson et al., 1978).

Hungate (1966) indicated that if turnover rate increases to the point that organisms cannot reproduce fast

enough to maintain the ruminal population, the microbiota is washed out. Average time for cell division must be approximately 69 percent of its turnover time to maintain the cell numbers in a continuous system (Hungate, 1966). Bergen et al. (1982) found that bacteria must have growth rates in the liquid and solid phases greater than 10 and 5 percent/h, respectively, to maintain adequate cell numbers.

Harrison et al. (1975), feeding sheep a diet of flaked maize and dried grass and changing dilution rate from 3.8 to 9.8 percent/h by intraruminal infusion of artificial saliva, found a 24 percent increase in Y(ATP). Using a diet based on dried grass, Kennedy and Milligan (1978) obtained an increase in Y(ATP) of 32 percent when dilution rate of sheep was changed from 6.8 to 13.6 percent/h. In the study by Harrison et al. (1975) with sheep, dilution rate increased microbial protein yield but in studies by Kennedy et al. (1976) and Kennedy and Milligan (1978), increasing dilution rate tended to lower this parameter. Harrison, cited by Harrison and McAllan (1980), observed that Y(ATP) increased from approximately 13.2 to 15.5 in sheep fed a semipurified diet containing urea as the sole nitrogen source if dilution rate increased from 3.3 to 7.7 percent/h. Cole et al. (1976) and Prigge et al. (1978) also have provided convincing evidence suggesting increasing

dilution rate increased microbial protein synthesis in steers.

According to calculations by Leng, cited by Leng and Nolan (1984), percentage of fermented organic matter incorporated into microbial cells increases as the Y(ATP) increases, but the percentage of fermented organic matter incorporated into VFA, CO_2 and CH_4 decreases. Leng and Nolan (1984) indicated that an improved microbial protein availability is of benefit to the host animal only if the animal is deficient in essential amino acids, because additional microbial protein output from the rumen is associated with a decrease of VFA production and absorption.

Effects of dilution rate on microbial protein synthesis may be associated with a dilution of the maintenance expenditure of bacteria, and/or reduction in the protozoa number and predation of bacteria (Prigge et al., 1978). Maalae and Kjeldgaard, cited by Bergen et al. (1982), found that at low dilution rates, bacterial cells had lower ribosome contents than at high dilution rates. However, Bergen et al. (1982) indicated that high cell densities and possible accumulation of inhibitor substances, rather than a growth-limiting substrate, may well control bacterial growth in the rumen. Hence, whenever the ruminal contents are diluted by any means, inhibitory product(s) and cell density decline, resulting in faster and more efficient bacteria growth.

Results of Crawford et al. (1980a), who used an in vitro continuous culture system controlling dilution rates at 7, 10, and 15 percent/h, and solid retention time (SRT) at 14, 22 and 29 h, demonstrated that Y(ATP) increased as dilution rate increased from 7 to 10 percent/h, but not at higher dilution rates. There were no significant effects of SRT on microbial efficiency, except when reduced from 22 to 14 h, but there were consistent trends toward increasing microbial efficiency with decreasing SRT. In this study, effect of dilution rate on microbial protein yield was positive but, in the case of SRT, it was negative. However, in another study (Hoover et al., 1982) in which different SRT's (16, 23, 28 and 40 h) were studied at a constant dilution rate (6.25 percent/h), SRT did not affect microbial protein yield.

There was a tendency for microbial protein yield/kg of organic matter digested to decrease as SRT increased, but this was significant only between 16 and 23 h.

Effect of Turnover Rate on the Ruminal Ecosystem

Several authors (Harrison et al., 1975; Isaacson et al., 1975) have indicated that dilution rate does not affect ruminal pH but this apparently depends on the agent used to alter dilution rate as well as on the composition of the diet.

With different experiments using intraruminal infusion of an artificial saliva solution to alter dilution rate,

Harrison et al. (1975) found that almost all induced changes in fermentation patterns occurred without significant alteration of ruminal pH. Using sodium chloride to modify dilution rate, Rogers et al. (1979) found that dilution rate reduced ruminal pH in steers fed a diet high in roughage, but there was no effect when animals were fed a diet high in concentrate. These results indicated that an alteration in salivary secretion rate in response to the hypertonic sodium chloride solution may differ according to the diet fed and may affect buffering capacity of the rumen.

Isaacson et al. (1975) did not find any effect of dilution rate on culture pH under in vitro conditions. However, Crawford et al. (1980b), using a special chemostat to control dilution rate and SRT, found that both variables affected culture pH. This effect was explained by changes in buffering capacity and (or) concentration of VFA produced as dilution rate and (or) SRT changed. Hoover et al. (1982), in contrast, found no effect of SRT (16 to 40 h) on culture pH using in vitro continuous cultures at a constant dilution rate.

As dilution rate is altered, bacterial population may change in profile or metabolism. Pure cultures often produce different end products at faster growth rates (Owens and Isaacson, 1977). With respect to the production of VFA, data obtained under in vitro (Isaacson et al., 1975) and in vivo conditions (Thomson et al., 1975, 1978; Harrison et

al., 1975) indicate that VFA production was not affected by dilution rate. In all of these experiments, VFA production was measured per animal or per mol of hexose fermented. However, Rogers and Davis (1982a) found an increased dilution rate resulted in increased VFA production, when VFA production was calculated per unit of dry matter consumed. These authors have pointed out that potential differences in substrate utilization and VFA production by ruminal microorganisms due to increases in dilution rates and osmotic pressure may be dependent on the type of diet fed. In vitro studies by Crawford et al. (1980b) showed that dilution rate and SRT had a positive but asymptotic effect on VFA production per unit of DM digested. Hoover et al. (1982) also found the same effect of SRT on VFA production per unit of DM digested. Crawford et al. (1980b) summarized data from different authors indicating values of VFA production between 4.23 to 6.49 and 4.4 to 6.0 mmoles per g of DM digested under in vitro and in vivo conditions, respectively.

Concentrations of VFA in ruminal fluid have been reported to decrease as dilution rate increased (Potter et al., 1972; Rogers et al., 1979; Rogers and Davis, 1982a, 1982b). Likely, it is due to a greater outflow of ruminal fluid as dilution rate increased. Several experiments have shown that VFA composition is affected by dilution rate. In general, when dilution rates increased, proportion of

acetate rose and proportions of propionate decreased (Harrison et al., 1975; Thomson et al., 1975; Rogers et al., 1979; Rogers and Davis, 1982a). However, effect on proportion of butyrate was not appreciable. These results do not agree with the in vitro results of Isaacson et al. (1975) who found that dilution rate enhanced the proportion of propionate. Czerkawaski and Breckenridge (1977) likewise found no effect of dilution rate on proportion propionate under in vitro conditions. Working with cows, Rogers et al. (1982) found no effect of dilution rate on total production of acetate and butyrate but a significant and negative effect on total production of propionate. These responses were obtained with a change in dilution rate from 4.2 to 5.5 percent/h.

Effect of dilution rate on proportions of different VFA depends on the type of diet fed. With diets high in concentrate, the effect of dilution rate follows the general pattern indicated above but with diets high in roughage dilution rate did not produce significant changes in ruminal fluid composition of VFA (Rogers et al., 1979; Rogers and Davis, 1982b). A possible explanation for the negative effect of dilution rate on propionate production was given by Thomson et al. (1978). Succinate, a precursor of propionate, decreases because increased dilution rates reduce the population of bacteroides and other ruminal bacteria that produce succinate, even though the population

of selenomonads that decarboxylate succinate is increased.

It is well established that there is a highly significant and positive correlation between the molar percentage of ruminal acetate and milk fat percent (Davis, 1979). High dilution rates could be important in preventing low-milk fat syndrome found in cows consuming high levels of concentrates (Davis and Brown, 1970).

Chalupa (1977) showed stoichiometric calculations of ruminal activity using data of Harrison et al. (1975). He found energetic efficiency of ruminal fermentation was lower at higher dilution rates because less metabolic hydrogen was recovered as VFA. In contrast, more alpha-linked glucose polymer escaped fermentation but total ruminal energy output was similar.

Considering solid turnover rate, some data (Crawford et al., 1980b) have indicated that molar proportions of acetate decreased significantly as solid flow rate increased while molar proportions of propionate and butyrate increased slightly. Results from another experiment using a constant dilution rate (Hoover et al., 1982) showed that the solid flow rate alone may not have major effect on the proportion of individual VFA's.

Prigge et al. (1978) obtained no effect of dilution rate on dry matter and protein digestibility in steers. In this study, the diets were isoenergetic and isonitrogenous

but proportion of soluble nitrogen was different among diets.

Crawford et al. (1980b) found that dry matter digestibility was enhanced significantly when dilution rate and SRT were increased in vitro. Digestibility coefficients of neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose and cellulose followed similar trends. At a dilution rate of 15 percent/h, there was no advantage in increasing SRT beyond 22 h. According to the authors, these data imply that for in vivo conditions, optimum dry matter digestibility might be attained by maintaining a relatively high dilution rate (11 to 15 percent/h), while keeping SRT between 22 and 30 h. Hoover et al. (1982) found that digestibility of DM and ADF increased significantly when SRT changed from 23 to 28 h. However, rate of digestion of DM decreased with increasing SRT, and was associated with a higher digestibility of starch and sugars at lower SRT's.

Increasing dilution rates through addition of mineral salts, Thomson et al. (1975) obtained lower apparent nitrogen digestibility but higher nitrogen retention in lambs fed mineral-supplemented diets compared to controls. Rogers and Davis (1982a) observed no significant effect of dilution rate on DM, OM, CP and ADF digestibility, though it tended to diminish. In contrast, digestion of starch was reduced as dilution rate increased. Bull et al. (1979) reported a

similar effect on starch digestion when ruminal turnover rate was increased by increasing dietary roughage content.

Wheeler (1979) suggested that an extensive starch flow to the intestine, along with insufficient buffering capacity, may result in suboptimal pH for maximum pancreatic amylase activity, which causes a reduction in starch digestion. Nevertheless, fecal starch digestion increased. Chalupa (1977) indicated that interaction between ruminal turnover rate and type of carbohydrate relative to digestion is complicated by particle size and density, distribution of particle sizes in the ration, and rate of ruminal fermentation. In another experiment with steers, Rogers and Davis (1982b) found no changes in ruminal DM disappearance with different dilution rates produced by solutions of sodium bicarbonate and sodium chloride infused intraruminally. Dacron bags suspended in the rumen for 12 and 24 hours were used to measure DM disappearance.

Effect of dilution rate on ruminal digestion of natural protein depends on the source of protein. Zinn, cited by Bull et al. (1979), found that ruminal digestion of protein decreased as dilution rate increased but the effect depended on the source of protein. In vitro studies have corroborated the negative effect of dilution rate on protein digestion (Bull et al., 1979). These authors also indicated that protein digestion in the total gastrointestinal tract decreased as dilution rate increased. This

could be related to a less time for enzymatic action, both in the rumen and intestines.

In vitro studies cited by Bull et al. (1979) have shown that dilution rate has a significant depressing effect on fiber digestion. Similarly, Bull et al. (1979) observed that dilution rate lowered cellulose digestion both in the rumen and total gastrointestinal tract. In contrast, Rogers and Davis (1982a) found no effect of changing dilution rate of ADF digestibility.

In a study with sheep consuming roughage diets and drinking an aqueous solution of sodium chloride (1.3 percent) or fresh water, intake of water was higher for animals drinking the hypertonic solution, which produced a higher dilution rate, compared to animals drinking fresh water (Potter et al., 1972). Other authors (Rogers et al., 1979) also found the same effect of dilution rate on water intake when steers were fed a diet high in grain or roughage, and ruminal infusions of sodium chloride solutions (5.5 and 11.1 percent) were used to change dilution rate. They found that increments in water intake produced by the osmotic agent were the same in both types of diets. This similar response across two vastly different dietary regimens suggests that water and electrolyte balance are highly regulated processes. In another experiment, using the same types of diets and osmotic agents, Rogers and Davis (1982b) obtained similar results, although animals

eating the high grain diet consumed less water than those eating the high roughage diet. Increase in water intake observed in these experiments may account, at least partially, for the increase in total ruminal fluid obtained when dilution rate was increased.

Rogers et al. (1979) did not find significant effects of dilution rate on DM intake in steers fed a high-grain or high-roughage diet and intraruminally infused with water and .5 kg NaCl in solution. Also, Rogers and Davis (1982a) found no effect of dilution rate on DM intake with steers fed a diet of 50 percent concentrate and 50 percent corn silage with sodium bicarbonate as the osmotic agent; rumen osmolality was increased up to 288 mOsm/kg of fluid. However, with a high roughage diet (Rogers et al., 1979), dry matter intake was reduced significantly when dilution rate was increased using 1.0 kg of sodium chloride infused intraruminally in solution to each animal daily. Authors indicated that high osmotic pressure could have depressed cellulose degradation since osmolality above 350 mOsm/kg of fluid has shown a negative effect on in vitro cellulose digestion (Bergen, 1972). Therefore, reduced cellulose degradation could have resulted in a greater ruminal fill. This may explain partially the lower DM intake observed using the high roughage diet. In addition, Bergen (1972) showed that when ruminal osmolality was above 400 mOsm/kg of fluid, feed intake decreased markedly in sheep. He

suggested the possibility of osmoreceptors in the rumen, limiting voluntary feed intake.

Factors Affecting Turnover Rate

Owens and Osaacson (1977) have classified the main factors affecting turnover rate of fluids and particles. Dilution rate is affected by fluid influx, which includes fluid intake, ruminal wall influx, salivary flow and ruminal volume. Solid turnover rate is determined by particle size, particle density and wettability. At the same time, many of these factors are affected by each other and others, such as salts, buffers, feed intake, dietary composition and processing of feed.

Ruminal Temperature

Normal ruminal temperature is considered to be 39° to 41°C, depending on location in the rumen, with higher temperatures in dorsal than ventral parts (Church, 1979a). Deviations from this range may affect ruminal microbial activity. One factor that significantly affects ruminal temperature is the temperature of fluids consumed (Dracy and Kurtenbach, 1968).

Studies by Kennedy et al. (1976) indicated that sheep maintained at an environmental temperature between 18 and 21°C were less efficient synthesizing microbial protein than those maintained at -1 to 1°C (47.9 vs 54.9 g N/kg OMD,

respectively), even though quantity of microbial DM or protein synthesized in the rumen did not differ between treatments. Increased efficiency of microbial production positively correlated with dilution rate, which was higher at lower temperatures. These results were later corroborated by Kennedy and Milligan (1978). Kennedy et al. (1976) pointed out that effects of temperature on rate of passage of digesta could be related to changes in ruminal motility. Levin, cited by Kennedy et al. (1976), implicated thyroid hormones in increased gut motility and enhanced gastric emptying observed in simple-stomached animals. Likewise, Westra, cited by Kennedy et al. (1976), found that concentration of serum triiodothyronine in sheep was increased when environmental temperature decreased from 21 to 1°C.

Feed Intake

Many authors (Minson, 1966; Grovum and Hecker, 1973; Grovum and Williams, 1977) have found that feed intake affects retention time of digesta in the rumen, and consequently, ruminal turnover rate. Using sheep fed dry pasture herbage, Minson (1966) found that as feed intake increased, ruminal retention time of digesta decreased. He measured retention time by directly removing the reticulorumen content. Grovum and Hecker (1973) found similar results for movement of digesta in the large intestine of sheep fed with lucerne chaff. In this experiment, retention

time was measured by killing the animal and determining intestinal contents. Using a marker technique, Grovum and Williams (1977) corroborated this effect of intake level on turnover rate. Owens et al., cited by Bull et al. (1979), showed that dilution rate increased as level of intake increased. Feeding animals at maintenance and twice maintenance, they obtained dilution rates of 3.0 and 5.3 percent/h, respectively. With data from different experiments involving both sheep and cattle, Evans (1981a) found a positive relationship between dilution rate and feed intake, and between solid turnover rate and feed intake (Evans, 1981b). Grovum and Williams (1977) have indicated that level of intake increases ruminal water volume, which is a factor not taken into account in many turnover measurements.

Dietary Forage Content

Several studies showed that content of forage in the diet affects dilution rate. With young steers receiving a diet of concentrate or hay cubes, Topps et al. (1968) found that volume of ruminal fluid did not differ between diets but outflow of fluid from the rumen was considerably higher with the hay cube diet. Using dairy cows fed with diets containing 10 and 60 percent of forage, Bauman et al. (1971) found that the ruminal fluid outflow was 4.3 and 9.7 liters/h, respectively. For cows fed the high-forage diet, 20.6 percent of ruminal fluid volume turned over every hour, while mean for cows fed the low-forage diet was 9.6 percent.

Hodgson and Thomas (1975) reported a similar effect of dietary roughage content on dilution rate in sheep. On the other hand, Evans (1981a) did not find a relationship between dilution rate and dietary forage content with either sheep or cattle. Nevertheless, he found a positive relationship between solid turnover rate and dietary forage content in sheep and cattle (Evans, 1981b). This author suggested that the effect of forage content on solid turnover rate may be associated with an increase in rumination and salivation. Differences in water consumption as affected by forage intake may be a factor also.

Inorganic Salts

Some salts increase osmotic pressure of ruminal fluid and this may play an important role in regulating dilution rate because both ruminal water flux and permeability are influenced by ruminal osmolality (Warner and Stacy, 1972). Sheep (Harrison et al., 1975) and steers (Rogers et al., 1979) receiving intraruminal infusions of an artificial saliva and sodium chloride solution, respectively, showed an increase in ruminal osmolality. This effect was associated with an increase in dilution rate. Other authors have corroborated the effect of inorganic salts on dilution rate using artificial saliva (Thomson et al., 1975, 1978). Also, data with diets high in concentrates (Rogers and Davis, 1982a, 1982b; Rogers et al., 1982) have shown a positive effect of sodium bicarbonate for increasing dilution rate.

Dilution rates for individual animals eating the same diet were not always similar and appeared to be modified by characteristics of individual animals and by type of diet previously fed (Harrison et al., 1975; Hodgson and Thomas, 1975). Harrison et al. (1975) found that ruminal infusion of mineral salts increased dilution rates only in those animals having low dilution rates on control diet.

Measurement of Ruminal Turnover Rate

Most techniques for measuring kinetics of ruminal digesta are based on utilization of external markers (Faichney, 1975; Van Soest, 1982). Criteria for an ideal marker have been discussed by Kotb and Luckey (1972). Most importantly, the marker must be strictly non-absorbed and move with the phase in consideration. There exists a list of markers proposed for the liquid and solid phase. Most commonly used markers include polyethylene glycol and chelates of trivalent Cr, Co and Fe with ethylenedinitrilotetraacetic acid (EDTA) or diethylenetrinitrilopentaacetic acid (DPTA) for the liquid phase, and stain, chelates of EDTA and tris-(1, 10-phenanthroline)-Ru, rate earth elements and mordanted fiber with Cr or Ce for the solid phase (Ellis and Lescano, 1982).

Faichney (1975) described procedure and mathematical treatment for the single dose time-sequence sampling technique, which has been used by many researchers to estimate

ruminal turnover rate of the liquid and solid phases. This technique is relatively simple and consists of introducing a single dose of the marker into the rumen and measuring its concentration in the rumen over time.

Several groups of researchers (Blaxter et al., 1956; Ellis et al., 1972; Grovum and Williams, 1973) have applied compartmental analysis techniques to fecal excretion time curves for several markers and have derived mathematical models to estimate ruminal turnover rate. However, interpretation of the various parameters of these models is not completely understood biologically.

Markers of Microbial Protein Synthesis

Various markers have been used to estimate microbial protein synthesis in the rumen or any part of the gastrointestinal tract. These include nucleic acids, purine and pyrimidine bases, amino acids and isotopes of elements present in microbial cells (Stern and Hoover, 1979). All techniques for estimating microbial protein using markers require determination of marker-to-N ratios in microbial cells and marker contents in digesta. The marker-to-N ratio is obtained by isolation of a representative sample of the microbial population, usually by differential centrifugation. Its determination often presents problems greater than those associated with subsequent chemical analysis of marker (Buttery and Cole, 1977).

Amino Acids as Microbial Protein Markers

Diaminopimelic acid (DAP) and aminoethylphosphoric acid (AEP) are the main amino acids used as microbial protein markers (Stern and Hoover, 1979). Diaminopimelic acid is present in the cell walls of bacteria but is not a constituent of bacterial protein. Although DPA is absent from plant material, traces can be detected in protozoa, probably due to engulfment of bacteria. One obvious problem of the DAP technique is that it only estimates bacterial protein, not protozoal protein. Also, any change in the proportion of bacterial proteins to bacterial cell walls (e.g., lysis of cells between rumen and digesta sampling sites) may introduce a bias in estimation of microbial protein. Another potential problem of this technique is the possibility of metabolism of DAP prior to digesta sampling (Buttery and Cole, 1977).

Aminoethylphosphoric acid is an amino acid present in the lipid fraction of protozoa and its assay, although difficult (Buttery and Cole, 1977), is one of the few methods of determining protozoal contribution to duodenal protein flow. Although AEP was reported to be absent in feeds and bacteria (Abou Akkada and Messmer, 1968), Ling and Buttery (1978) found AEP in some concentrate feed hydrolysates. Czerkawaski, cited by Buttery and Cole (1977), also found AEP in bacteria.

Nucleic Acid as Microbial Protein Markers

Ellis and Pfander (1965) and Smith et al. (1968) found a constant microbial nucleic acid-to-protein ratio under a variety of dietary conditions and suggested the utilization of nucleic acids for estimating microbial protein. Several authors have used RNA to estimate amount of microbial protein in ruminal fluid and digesta (Smith and McAllan, 1970; Smith et al., 1978; Arambel et al., 1982).

Such a method relies on the assumption that all dietary nucleic acids are broken down in the rumen and nucleic acids leaving the rumen are of microbial origin. Using in vitro and ruminal infusion techniques, several studies (McAllan and Smith, 1968; McAllan and Smith, 1973) indicated that DNA and RNA were broken down rapidly. However, there were doubts as to whether this was true entirely, and microbial protein flow may have been over estimated, especially when large portions of dietary nucleic acids were insoluble (Buttery and Cole, 1977). Another assumption of this method is that a reliable microbial nucleic acid-to-protein ratio exists (Schelling et al., 1982). They summarized data from literature indicating a constant microbial nucleic acid-to-protein ratio. However, Smith and McAllan (1974) reported variability in this ratio.

Reliable representativity of digesta samples for assessing nucleic acids is another factor affecting usefulness of

this method. In general, it is accepted that more uniform samples can be collected from the duodenum than from the abomasum, since digesta may suffer stratification in the abomassum but its flow is laminar in the duodenum (Schelling et al., 1982). However, a major concern with duodenal sampling is the possibility of digestion of nucleic acids and absorption of their constituents (Schelling et al., 1982).

Analytical procedures for the determination of nucleic acids also limit their utilization. The procedure of McAllan and Smith (1969) is very laborious. Simple and quicker procedures were introduced by Ling and Buttery (1976), and more recently, by Zinn and Owens (1980), but more information is required regarding their precision and accuracy. This analytical limitation led to consideration of purine and pyrimidine bases as markers of microbial protein. Preliminary in vitro and in vivo studies (Koenig et al., 1979, 1980) under different microbial growth conditions indicated a potential use of certain purine and pyrimidine bases, especially cytosine and adenine, as microbial protein markers. Cytosine has an advantage over adenine due to its rapid degradation by ruminal microbes (Koenig, 1980), which reduces errors in estimation of microbial protein produced by dietary cytosine or any purine and pyrimidine base under consideration (Schelling et al., 1982).

Isotopes as Microbial Protein Markers

Of the isotopes used to measure microbial protein, ^{35}S has been used most frequently (Stern and Hoover, 1979). This technique consists in labelling a microbial fraction with ^{35}S and determining the dilution of the specific activity of a sulfur component in both digesta and the microbial fraction (Buttery and Cole, 1977). The basic premise of this method is that all the sulfur incorporated into microbial protein first passes through the free H_2S pool. However, there is direct incorporation of dietary S-amino acid into microbial protein, although there is disagreement about extent of incorporation (Stern and Hoover, 1979).

Labeled phosphorus, ^{33}P, was proposed by Bucholtz and Bergen (1973) when they observed a good correlation between phosphorous uptake and its incorporation into microbial phospholipids. Then Van Nevel and Demeyer (1977) expanded this approach to the utilization of ^{32}P for estimating microbial protein. This method is based on many assumptions such as that all the P incorporated into microbial protein comes from the labeled inorganic precursor pool, specific activity of the intracellular precursor is used only for microbial growth, there is no degradation of nonlabeled cells, and cell composition remains constant during growth (Stern and Hoover, 1979). Some data have indicated that not all of these assumptions are true, especially the latter two, which likely limits the value of this technique (Stern and Hoover, 1979).

Another isotope used to estimate microbial protein is ^{15}N. This method is based on the incorporation of N from ammonia into microbial protein and does not account for the microbial protein synthesized directly from amino acids and peptides (Stern and Hoover, 1979). A great advantage of ^{15}N is that it deals with N, but as a routine technique for assessing microbial protein, Buttery and Cole (1977) suggest it has too many complications.

Comparisons of Methods for Estimating Microbial Protein

Using data from literature, Theurer (1982) concluded that estimates of microbial protein in abomasal or duodenal digesta were often lower when DAP was used compared to estimate based on RNA and ^{35}S. These data also showed that RNA gave higher estimates of microbial protein than ^{35}S. The usual explanation for the low DAP-based values is the inability of the DAP method to estimate protozoal protein (Theurer, 1982), although it has been suggested that the contribution of protozoal N to duodenal N is relatively small (Weller and Pilgrim, 1974). Microbial protein values based on RNA may be higher than those estimated from other markers because of an overestimation of the microbial fraction due to incomplete ruminal degradation of dietary RNA (Theurer, 1982). This was demonstrated by Smith et al. (1978) using ^{35}P-labeled RNA. Other studies (Walker and Nader, 1975; Ling and Buttery, 1978) indicated that, with

few exceptions DAP, RNA and ^{35}S ranked microbial protein in digesta in the same order. Smith and McAllan (1974) concluded that when total microbial values are required, method of choice becomes either RNA or ^{35}S. Also, when microbial protein estimates of a more general and comparative nature are required, the use of RNA would probably be adequate. However, when more accurate estimates are required, ^{35}S would be more appropriate.

A comparison of nucleic acids, DAP and amino acid profiles as microbial protein markers indicated that total nucleic acid gave best estimates of microbial protein, followed by amino acid profiles and, finally, DAP (Nikolic and Jovanoic, 1973). Kennedy and Milligan (1978) used ^{15}N and ^{35}S for estimating microbial protein in sheep and found 10 percent lower microbial protein with ^{15}N than with ^{35}S.

Sodium and Potassium in Nutrition and Physiological Processes in Ruminants

Chemically, sodium is similar to potassium and they are associated in many physiological reactions. In the animal, sodium occurs largely in extracellular body fluids and bones and potassium intracellularly, mainly in muscle and nervous tissues (Clanton, 1980). Their essential functions are maintenance osmotic pressure, regulation of acid-base equilibrium and control of water metabolism in body tissues (Underwood, 1981). Many enzymes have a specific

requirement for potassium, and this mineral also acts with other ions such as sodium, magnesium and calcium in influencing enzyme activities (Underwood, 1981).

Potassium deficiency, with rare exceptions, is not believed to be a problem in ruminants (Church, 1979b). In contrast, extensive areas of sodium deficiency in livestock occur in many parts of the world (Underwood, 1981). Requirements suggested by the NRC (1978) for sodium and potassium in dairy cows are .18 and .80 percent, respectively. It is very possible that requirements for potassium and sodium are partially related to proper ruminal function rather than to requirements of body tissues and functions (Hemken, 1980).

Data from Florida has shown that heat stress increases the K requirement of dairy cows. Beede (1981) suggested that an increased turnover rate of body K, presumably due to increased sweating during hyperthermia, could result in a K deficiency. When dietary K content was increased from .66 to 1.08 percent (Beede et al., 1984), there was an increase in feed intake (7 percent) and daily milk production (12 percent) during heat stress. This effect of K on intake and milk production under heat stress was corroborated with cows consuming K at levels of .8 and 1.2 percent in the diet (Beede et al., 1983b). Under conditions of no heat stress, an interaction between sodium and potassium was observed in dairy cows (Beede et al., 1983b). It was concluded that changing dietary amounts and proportions of sodium and

potassium has an effect on milk yield which cannot be attributed solely to an associated higher feed consumption.

A cost-benefit analysis conducted on the basis of Florida studies (Beede et al., 1983a) indicated that the addition of K as potassium chloride and Na in chloride or bicarbonate forms at 1.5 to 1.8 percent and .4 to .6 percent dry matter, respectively, can increase net profit per cow per day compared to formulating rations more near current NRC (1978) requirements.

Another important aspect of sodium and potassium is their role in the cation-anion balance since different authors have found that dietary cation-anion balance does have an influence on ruminant performance (Nicholson and Cunningham, 1961; Wise et al., 1965). Dietary cation-anion balance is associated primarily with sodium, potassium and chloride because other cations (calcium and magnesium) and anions (phosphate and sulfate) have only a small influence on total physiological acid-base balance (Wheeler and Hruska, 1981). Sodium and potassium work as alkalogenic elements and chloride as an acidogenic element.

Cation-anion balance is important for several physiological functions of the body such as enzymatic reactions, conduction of nerve impulses and maintenance of osmotic integrity of body fluids. Based on the results of several authors, Wheeler and Hruska (1981) indicated that a cation-anion balance above 50 mEq/100 gm diet appeared to have

little benefit in either beef or dairy cattle feeding programs, and for cows most often was around 10 mEq/100 gm diet. However, relationships between dietary cation-anion balance and lactational performance have not been well defined.

Maximum tolerable concentrations of sodium established for cows and other livestock are 4 and 9 percent sodium chloride, respectively. For potassium, the maximum tolerable concentration for cattle is set at 3 percent (NRC, 1980). However, high potassium intake may lower magnesium absorption, which is associated with grass tetany (Underwood, 1981).

Sodium and Potassium Effects on Ruminal Microbes

Many studies (McLeod and Snell, 1948; Hubbert et al., 1958; Bryant et al., 1959; Caldwell and Hudson, 1974) have shown that sodium and potassium are necessary for growth and activities of many different microorganisms present in the rumen. Many metabolic and physiological functions of potassium are well documented for bacteria (Caldwell and Hudson, 1974). Studies conducted with disrupted cell preparations of ruminal bacteria showed that most species contain enzymes requiring potassium as a cofactor (Joyner and Baldwin, 1966). Duguid and Wilkinson (1954) suggested that synthesis of polysaccharide is inhibited specifically by a deficiency of potassium. Function(s) of sodium in the

physiology and metabolism of these organisms seem to be less well understood (Caldwell and Hudson, 1974).

In an in vitro study with different ruminal species of bacteria, Caldwell and Hudson (1974) found differences in the minimum Na concentration required to support rapid and abundant growth. Optimum growth of these species was achieved at Na concentrations similar to those found in the ruminal fluid (60 to 120 mM). Concentrations of Na higher than optimum did not affect bacterial growth. They indicated that differences in the quantitative Na requirements among species should not substantially affect their growth in the rumen, since the Na concentration normally found in ruminal fluid is more than adequate to support abundant growth of all of Na requiring species, and the growth of species that require relatively small amounts of Na apparently is unaffected by Na concentrations much greater than the minimal concentration that supports rapid and abundant growth. With bacteroides amylophylus, maximum growth was obtained with a Na concentration of 60 mM (Caldwell et al., 1973). Working in vitro with ruminal microorganisms, Hubbert et al. (1958) observed a curvilinear effect of Na on cellulose digestion but there was no significant increase in cellulose digestion with Na concentrations between 1 and 102 mM.

Working with many bacterial species Lester (1958) found that K was necessary for bacterial growth. Different

authors (Hubbert et al., 1958; Caldwell and Hudson, 1974) have found microbial requirements for K to be substantially lower than those for Na. With bacteroides succinogenes, maximum growth was obtained with concentrations of K ranging from 3 to 10 mM (Bryant et al., 1959). However, with bacteroides amylophylus (Caldwell et al., 1973), maximum growth was obtained with a K concentration of 1.3 mM. Further addition of K to a concentration of 78 mM did not increase growth yield. Also they found a significant interaction between Na and K. At low Na levels, high levels of K were necessary for maximum growth. With ruminally washed bacteria, Hubbert et al. (1958) found a curvilinear effect of K on cellulose digestion. Maximum response was obtained with K concentrations between 13 to 51 mM. They indicated if K was present in concentrations of 1.2 mM or less, Na may inhibit metabolic activity of organisms by competing with potassium for the active sites of the potassium-specific enzyme systems. Interactions between K and Na found in studies of cellulolysis were rather complex, and toxic effects observed with additions of both Na and K were probably due to excessive osmolality of the medium. Durand and Kawashima (1980) concluded that between .5 to 1.5 g/liter (12.8 to 38.5 mM) of available K is required for optimum fermentation in the rumen, but it has not been established how much was required by the micro-organisms per se and how much optimizes external physiochemical

factors. Studying the effect of K (0 to 1 M) on in vitro utilization of glucose by ruminal bacteria, Khan et al. (1969) found as the level of K increased, the utilization of glucose and production of VFA decreased.

There are few reports available describing the effect of potassium on utilization of urea or other sources of NPN by microorganisms. Apparently, this topic has not been investigated with respect to sodium. Burroughs et al. (1951) studied the effect of molasses ash, which has a high content of potassium, on in vitro utilization of urea by ruminal microorganisms. They found a positive effect of molasses ash on urea utilization. In contrast, Khan et al. (1969) studied the effect of potassium (0, 200, 500 and 1000 mmol KCl/liter) on in vitro rumen microbial activity and found that added potassium depressed urea utilization. They concluded that excessive potassium inhibited microbial activity.

Zembayashi, cited by Durand and Kawashima (1980), reported that protein and vitamin B_{12} synthesis were both stimulated by the addition of Na and K. However, Durand et al., cited by Durand and Kawashima (1980), found that in vitro utilization of urea was not modified by increasing level of K from 480 to 1000 mg/liter (12.3 to 25.6 mM). They suggested these discrepancies in microbial response to K were certainly due to differences in medium composition.

Utilization or Urea

Ability of ruminants to convert non-protein nitrogen (NPN) to protein was demonstrated almost a century ago (Loosli and McDonald, 1968). Since then, a large number of experiments have been conducted with the purpose of replacing at least part of dietary true protein with NPN.

Several NPN compounds have been studied as feed ingredients for the bovine, including ammonium acetate, ammonium bicarbonate, ammonium carbonate, ammonium lactate, biuret, glutamine and urea (Loosli and McDonald, 1968). In vitro, Belasco (1954) studied biological availability of many NPN sources and found urea was the most readily available for ruminants. Modern chemical and microbiological techniques have demonstrated that urea is converted into amino acids and protein in the rumen (NRC, 1976) and since 1950, urea has been included as an ingredient in diets of some ruminants (NRC, 1976).

During the last 20 years, excellent reviews regarding the utilization of urea by ruminants have been published (Loosli and McDonald, 1968; Chalupa, 1972; Henderickx, 1976; Huber and Kung, 1981), which cover both basic and applied nutritional aspects. A general recommendation for urea feeding in ruminants is that it be limited to 30 percent of the crude protein requirement at which level it does not have a deleterious effect on animal response (NRC, 1976). For lactating cows, 1 percent urea in the total dietary

dry matter, or 200 to 250 g/day, are adequate general guidelines (NRC, 1976).

Efficiency of urea utilization depends on different physiological and metabolical processes that transform urea into microbial protein in the rumen (NRC, 1976). Ruminal hydrolisis of urea into ammonia occurs too fast, which affects its utilization (Chalupa, 1972). Reduction of urease activity with different chemical compounds has been proposed to reduce the rate of urea hydrolysis (Steeter et al., 1969; Tillman and Sidher, 1969) but has had little practical success.

Ingestion of urea at periodical intervals was suggested as another means to increase its utilization. However, results obtained by different researchers are inconsistent (Knight and Owens, 1973; Streeter et al., 1973; NRC, 1976).

Quantity and quality of dietary preformed protein and energy are very important factors in utilization of urea (Loosli and McDonald, 1968). With respect to nitrogen metabolism in the rumen, utilization of urea by microorganisms depends on ammonia concentration in the ruminal fluid which, at the same time, depends on ruminal protein degradation (Chalupa, 1972). In vitro and in vivo studies with cattle (Satter and Slyter, 1974; Huber and Kung, 1981) indicated that maximum microbial growth is reached at a specific ammonia concentration in the ruminal fluid. Above this concentration, urea and other NPN sources were not

utilized for microbial protein synthesis. Satter and Slyter (1974) reported from an in vitro study that ammonia concentration for maximum microbial growth was 5 mg/100 ml fermentation fluid. However, in vivo studies (Huber and Kung, 1981) suggested ammonia concentrations between 10 and 29 mg/100 ml ruminal fluid for maximum microbial growth. According to Satter and Roffler (1975), the optimum level of ammonia for microbial growth is obtained with a level of dietary crude protein equivalent to 11 to 15 percent, dry basis.

Urea is not utilized as well when it is fed alone with either forages or easily fermentable sugar as when it is included with either cereal grains or starch (NRC, 1976). This is related to the rate of degradation in the rumen which, in the case of starch, more closely parallels rate of ammonia utilization by microorganisms (NRC, 1976). Amount of NPN incorporated into microbial protein also depends on amount of dietary energy (Satter and Roffler, 1975). These authors developed a model for predicting NPN utilization by cattle which relates ruminal ammonia concentrations to dietary crude protein and total digestible nutrients.

A gradual adaptation of animals to urea has been proposed as another mechanism to improve utilization of urea (Huber and Kung, 1981). However, other investigators have failed to produce clear proof of this effect (NRC,

1976). Mechanisms for adaptation are not clear and apparently do not include microbial adaptation (Huber and Kung, 1981). Stimulation of the urea cycle and accustoming cattle to urea diets, resulting in improved animal acceptance, are thought to be operative as mechanisms of urea adaptation (NRC, 1976).

It has been demonstrated that a specific nitrogen-to-sulfur ratio in the diet is required for maximum microbial utilization of urea (Loosli and McDonald, 1968). For cattle, this appears to be between 12:1 and 15:1 (Huber and Kung, 1981).

Another mean of increasing urea utilization may involve changing ruminal kinetics as it was found that dilution rate improved yield and efficiency of microbial protein synthesis (Harrison et al., 1975; Isaacson et al., 1975; Kennedy and Milligan, 1978). Results by Harrison, cited by Harrison and McAllan (1980), pointed toward a positive effect of dilution rate on microbial utilization of urea, although the experiment was not designed to study that point. Using sheep and a purified diet containing urea as the only source of nitrogen, he obtained Y(ATP) values of 13.3 and 15.5 with dilution rates of 3.3 and 7.5 percent/h, respectively.

A primary objective of the research reported herein (Chapter 4) was to study effects of ruminal kinetics on microbial utilization of urea-nitrogen. Preceding that

were experiments designed to characterize the influence of varying concentrations of potassium on urea utilization and apparent microbial N utilization in mixed ruminal cultures in vitro (Chapter 3).

CHAPTER 3

EFFECT OF POTASSIUM (KCl) ON APPARENT UTILIZATION OF UREA-NITROGEN BY MIXED CULTURE OF RUMINAL MICROORGANISMS IN VITRO

Introduction

In vitro studies with different microbial species have demonstrated that potassium (K) is important for microbial growth (Duguid and Wilkinson, 1954; Hubbert et al., 1958; Caldwell et al., 1973). Apparently, potassium requirements vary with microbial species. Durand and Kawashima (1982), in a review, concluded that .5 to 1.5 g available K per liter of digesta was required for optimum ruminal fermentation.

Aside from general statements that a deficiency of potassium affects microbial growth (Hubbert et al., 1958; Bryant et al., 1959; Caldwell et al., 1973) and consequently, utilization of other nutrients, there is little information regarding effect of potassium on utilization of nonprotein nitrogen (i.e., urea) by microorganisms. Durand and Kawashima (1982) summarized in vitro data which indicated that utilization of urea was not modified by increasing concentrations of K from .48 to 1.0 g/liter fermentation fluid. In another in vitro study, Khan et al. (1969) found that high concentrations of potassium depressed urea utilization.

Theoretically, potassium may affect utilization of urea by ruminal microorganisms, directly at the microbial level and (or) indirectly, through a modification of the ruminal ecosystem. The objective of the present study was to characterize effects of potassium (KCl) on in vitro utilization of urea by mixed cultures of ruminal microorganisms.

Materials and Methods

Study consisted of two experiments in which effects of different concentrations of added potassium (K) and urea (U), and different time intervals of fermentation (T) on apparent microbial nitrogen (N) utilization were studied. In both experiments, a 4x4x5x5 factorial arrangement of treatments was used. In Experiment 1 concentrations of K and U were 0, .87, 1.74 and 2.62 g added K/liter of fermentation fluid (0, 1.67, 3.35 and 5.04 g KCl) and 0, 53, 106 and 159 mg added U/liter (0, 22.9, 45.7 and 68.6 mg N). Fermentation time intervals were 2, 4, 6, 8 and 10 h. Each treatment was replicated five times on different days, with one observation per treatment each time.

In Experiment 2, K and U treatment additions were changed to 0, 1.51, 3.02 and 4.53 g added K/liter (0, 2.90, 5.80 and 8.73 g KCl) and 0, 143, 286 and 429 mg added U/liter (0, 60.8, 121.6 and 182.4 mg N), respectively. Fermentation intervals were same as in Experiment 1. Trial

was replicated four different days, with one observation per treatment on each day.

Source of ruminal fluid was a fistulated Jersey cow. During Experiment 1 (January through April) the cow received 11.0 kg of a complete mixed diet (Table 1) at 0900h daily. During Experiment 2 (May through July) intake dropped to 8.6 kg daily, probably due to warmer environmental temperatures. Diet was formulated with crude protein (CP), total digestible nutrients (TDN), potassium (K) and sodium (Na) contents of 12, 68, .4 and .2 percent, dry basis. Potassium was supplied at a level below that recommended by the NRC (1978) to maintain a low concentration in ruminal fluid. Before conducting preliminary trials and the actual experiments, the cow adapted to the diet for 4 wk.

In vitro fermentation procedure was based on the method described by Johnson (1966). Ruminal fluid was taken between 0700 and 0800 h from ventral part of the rumen, strained through four layers of cheesecloth and transported to the laboratory in a thermos jug (39°C). It was transferred within 5 to 10 min to a 4 liter culture flask containing buffer solution and situated in 39°C water bath. Ruminal fluid was taken at this time (prior to the daily feeding) in order to obtain an inoculum low in nitrogen and potassium contents. Fermentation fluid was comprised of one volume of ruminal fluid and four volumes of buffer. Fermentation fluid was flushed continuously with gaseous CO_2 and dosed

TABLE 1. COMPOSITION OF THE DIET FED TO RUMINAL FLUID DONOR COW

Item	%, dry basis
Crimped yellow corn	38.70
Brewer's dried grains	30.00
Cottonseed hulls	30.00
Urea	.50
Limestone	.33
Potassium chloride	.17
Trace mineral salt and vitamins	.20

randomly into polyethylene fermentation tubes (50 ml) within 20 min using a 50 ml repipet. Volume of fermentation fluid per tube was 20 ml. Inoculated tubes were purged immediately with gaseous CO_2 to maintain anaerobic conditions, and fitted with rubber stoppers with hypodermic needles to release fermentation gases.

Fermentation was run at 39°C using a water bath, hand swirling tubes every hour during the fermentation period. Fermentation was arrested with a saturated solution of $HgCl_2$ (1 ml per 100 ml of fermentation fluid), and tubes were centrifuged immediately at 3000 x g in a refrigerated centrifuge for 10 min. Supernatant was decanted and stored at 4°C for chemical analysis.

Buffer used was a modification of McDougall's artificial saliva (McDougall, 1948) with 20 percent more buffering capacity (Koenig, 1980) and without potassium salts (Table 2). The pH of individual culture tubes was adjusted to between 6.8 and 6.9 every 2 h with about .05 ml of a solution of Na_2CO_3 (20% w/v) or H_3PO_4 (50% v/v) (Moore et al., 1962). After pH adjustment each fermentation tube was flushed immediately with CO_2 and returned to the 39°C water bath.

Substrate was a semipurified mixture (75 percent cornstarch and 25% cellulose) included at a constant amount of .3 g/fermentation tube. This quantity was selected from preliminary trials to provide enough energy for microbial

TABLE 2. COMPOSITION OF THE BUFFER

Ingredient	g/liter
Na_2HCO_3	11.700
Na_2HPO_4	4.400
NaCl	.470
$CaCl_2$	.004
$MgSO_4$	.012

growth under conditions of these experiments. Solutions (1 ml) containing potassium and urea to give final prescribed treatment concentrations were added to fermentation tubes before inoculation. Nitrogen and NH_3-N were determined by autoanalyzer method (Technicon Industrial System, 1978), and sodium and potassium were analyzed by atomic absorption spectrophotometer (Perkin-Elmer Model 5000, Norwalk, CT). For analysis of NH_3-N, sodium and potassium, samples were deproteinated with approximately 30 mg sulfosalycylic acid/ml, and centrifuged at 300 x g for 10 min in a refrigerated centrifuge.

Apparent microbial nitrogen utilization (AMNU) was the dependent variable and was calculated using a computational procedure similar to that previously described by Hoover et al. (1982).

$$\text{AMNU} = (\text{UREA-N}) + (NH_3\text{-N})_i - (NH_3\text{-N})_r$$

where

AMNU = apparent microbial nitrogen utilization, mg N/100 ml fermentation fluid

UREA-N = nitrogen provided from U, mg/100 ml

$(NH_3\text{-N})_i$ = ammonia nitrogen at T = 0, mg/100 ml

$(NH_3\text{-N})_r$ = ammonia nitrogen at the end of fermentation interval, mg/100 ml

Statistical analysis was carried out by method of analysis of variance using general linear model procedures

of SAS (SAS Institute, Inc., 1982). The statistical model used was

$$Y_{ijkl} = U + R_i + K_j + R_iK_j + U_k + R_iU_k + K_jU_k + R_iK_jU_k + T_l + R_iT_l + K_jT_l + R_iK_jT_l + U_kT_l + R_iU_kT_l + K_jU_kT_l + E_{ijkl}$$

where

U = overall mean

R_i = fixed effect of ith day of ruminal fluid collection

K_j = fixed effect of jth added potassium

R_iK_j = interaction of ith day of ruminal fluid collection and j^{th} added potassium

U_k = fixed effect of kth added urea

R_iU_k = interaction of ith day of ruminal fluid collection and j^{th} added urea

K_jU_k = interaction of jth added potassium and k^{th} added urea

$R_iK_jU_k$ = interaction of ith day of ruminal fluid collection, j^{th} added potassium and k^{th} added urea

T_l = fixed effect of lth fermentation interval

R_iT_l = interaction of ith day of ruminal collection and l^{th} fermentation interval

K_jT_l = interaction of jth added potassium and l^{th} fermentation interval

$R_iK_jT_l$ = interaction of ith day of ruminal fluid collection, j^{th} added potassium and l^{th} fermentation interval

U_kT_l = interaction of kth added urea and l^{th} fermentation interval

$R_iU_kT_l$ = interaction of ith day of ruminal fluid collection, k^{th} added urea and l^{th} fermentation interval

$K_jU_kT_l$ = interaction of jth added potassium, k^{th} added urea and l^{th} fermentation interval

E_{ijkl} = residual effect

Orthogonal contrasts were used to test linear and quadratic terms of two-way interactions.

Results and Discussion

Experiment 1

Concentrations of K and NH_3-N and pH of ruminal fluids harvested to prepare inocula varied from 1.10 to 1.25 g/liter, 2.7 to 8.3 mg/100 ml, and 5.8 to 6.2, respectively. These values generally were low, probably because collection was about 23 h after the last feeding. Concentrations of K and NH_3-N in fermentation fluids were below those recommended by Durand and Kawashima (1980) and Satter and Slyter (1974) for maximum ruminal microbial growth.

Means of main effects of R, K, U and T on apparent microbial nitrogen utilization (AMNU) are in Table 3. Apparent microbial nitrogen utilization ranged from 1.25 to 6.78 mg/100 ml of fermentation fluid. Analysis of variance is in Table 4. All main effects (R, K, U and T) were significant as well as two and three-way interactions ($P>.001$), with exceptions of R*K*U and K*U*T ($P>.10$). It was assumed ruminal fluids and thus fermentation fluids would be somewhat variable in chemical and microbial composition from day to day; therefore, the three-way interactions were included to account for additional variability, though their biological interpretation may be difficult.

Means (Table 3) for effect of R on AMNU varied from 3.47 to 4.47 mg/100 ml. Responses to K, U and T were affected by day of ruminal fluid collection (i.e., R*K, R*U and R*T interactions, $P<.001$). These effects likely were a result of either variations in microbial or chemical composition of ruminal fluids. Even though time of feeding and composition of diet fed the donor cow were constant, other environmental conditions may have produced changes in ruminal microbial population, both in number and species (Hungate, 1966).

Though day of ruminal fluid collection (R) and R*K, R*U and R*T interactions were significant (Table 4), trend of each main effect, K, U and T, across ruminal fluids

TABLE 3. MEANS OF MAIN EFFECTS OF R, K, U AND T ON APPARENT MICROBIAL NITROGEN UTILIZATION (EXPERIMENT 1)

Independent Variable	AMNU[a]	Independent Variable	AMNU[a]
Ruminal Fluid (R)		Added Potassium (K) g/liter	
1	3.82	0	4.19
2	4.47	.87	4.10
3	4.14	1.74	3.99
4	4.48	2.62	3.90
5	3.47		
Standard Error	.024		.022
Urea (U) mg/liter		Fermentation Interval (T), h	
0	1.25	2	1.60
53	3.18	4	3.69
106	4.97	6	4.87
159	6.78	8	5.04
		10	5.04
Standard Error	.022		.024

[a]Apparent microbial N utilization, mg/100 ml of fermentation fluid.

TABLE 4. ANALYSIS OF VARIANCE (EXPERIMENT 1)

Source	d.f.	Mean Squares
Ruminal fluid (R)	4	.682***
Added K concentration	3	.061***
Urea concentration (U)	3	22.416***
Fermentation interval (T)	4	6.992***
R*K	12	.009***
R*U	12	.041***
R*T	16	.111***
K*U	9	.008***
K*T	12	.021***
U*T	12	.794***
R*K*U	36	.002[NS]
R*K*T	48	.005***
R*U*T	48	.014***
R*U*T	36	.002[NS]
Residual	144	.002

[a]For apparent microbial N utilization, mg/100 ml.

[NS](P>.10)

***(P<.001)

were similar and in the same directions. Being able to quantify the effects of R and interactions with R allowed us to more precisely estimate effects of primary variables of interest, K, U and T, and their interactions.

Increasing K negatively affected apparent microbial nitrogen utilization on each ruminal fluid collection day resulting in a slight decrease from 4.19 to 3.90 mg/100 ml of fermentation fluid as K increased from 0 to 2.62 g/liter of fermentation fluid ($P<.001$). However, response to K was slightly different on different ruminal fluid collection days (R*K interaction, $P<.001$). Overall, these results suggested that concentrations of K in fermentation fluids were probably adequate for maximum microbial growth in this in vitro system. Apparent microbial nitrogen utilization response to U increased linearly in each ruminal fluid and as a main effect increased from 1.25 to 6.75 mg/100 ml as U increased from 0 to 159 mg/100 ml. There was a R*U interaction ($P<.001$), indicating AMNU response to increasing U was not identical on each ruminal fluid collection day. In each ruminal fluid, AMNU response over fermentation time interval was curvilinear (Figure 1), reaching a plateau after 6 h. Pooled fermentation AMNU over all ruminal fluid collection days increased from 1.60 to 5.04 mg/100 ml as T increased from 2 to 6 h. Plateau effect after 6 h may have been produced by a shortage of nutrient(s) or an accumulation

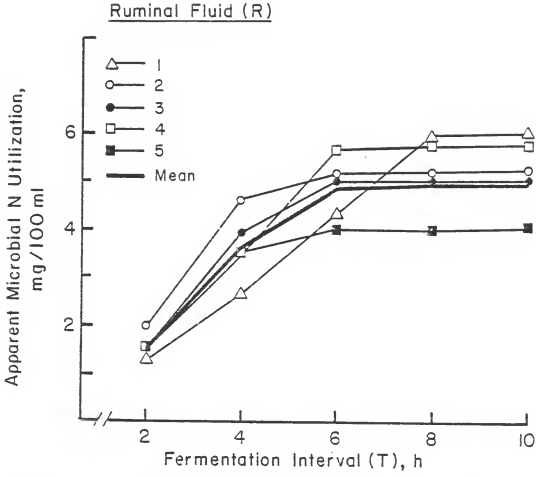

Figure 1. Interactive effect of T and R on apparent microbial N utilization (Experiment 1).

of fermentation end-products, which inhibited microbial growth.

The K*U interaction is indicated in Figure 2. Orthogonal contrasts indicated that the linear*linear term was significant ($P<.01$) and accounted for 80 percent of the variability associated with K*U. Trend of effect of K on AMNU was similar across U concentrations. Most of the slight decrease in AMNU resulting from increasing K concentrations was observed at the two highest concentrations of U. Little effect of K on AMNU at zero U concentration was noted, which may be associated with a deficiency of N to promote microbial growth. However, extent to which K affected AMNU (slope of linear regression of AMNU on K over U) increased as U increased. Apparent microbial nitrogen utilization per g added K was -.029, -.071, -.145 and -.190 at 0, 53, 106 and 154 mg added U, respectively. Positive effect of U on AMNU was similar at each concentration of K, although extent of apparent nitrogen utilization decreased slightly from .036 to .033 mg/mg urea, as K concentrations increased from 0 to 2.62 g/liter.

There was a K*T interaction (Figure 3). A greater negative effect of K on AMNU was shown at 2 and 4 h fermentation intervals than at 6 h of fermentation, and little or no effect at 8 and 10 h. Likewise, effect of T on AMNU was similar at different concentrations of K (T*K, $P<.001$, Figure 4). Generally, apparent microbial nitrogen

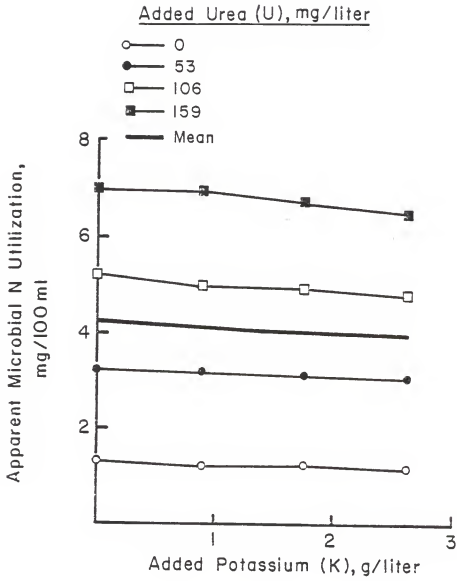

Figure 2. Interactive effect of K and U on apparent microbial N utilization (Experiment 1).

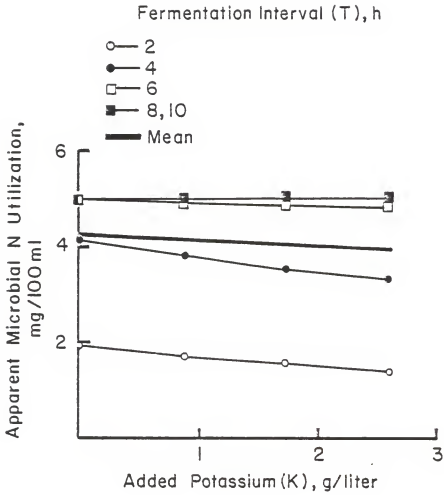

Figure 3. Interactive effect of K and T on apparent microbial N utilization (Experiment 1).

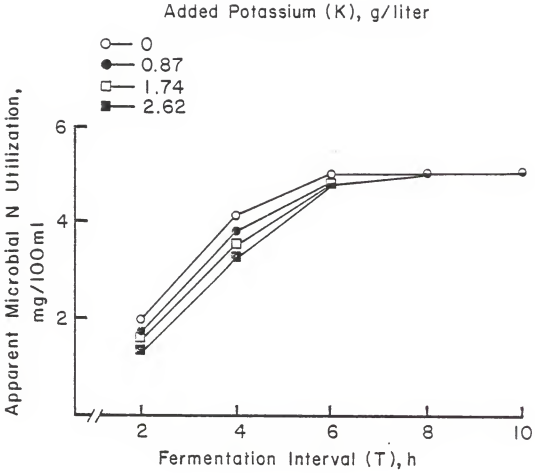

Figure 4. Interactive effect of T and K on apparent microbial N utilization (Experiment 1).

utilization increased as T increased but reached a plateau at T greater than 6 h (Figure 4). However, 59 percent of the variability associated with the interaction (T*K) was accounted for by the linear*linear term.

At each fermentation time, AMNU increased as U concentration increased (Figure 5) and there was a U*T interaction (P<.001). Results showed a tendency for a curvilinear response to U at 2 and 4 h fermentation time, but response was more linear at 6, 8 and 10 h. Linear*linear term accounted for 83 percent of variability associated with the U*T interaction, but linear*quadratic term also was significant (P<.001). Apparent curvilinear response of AMNU to added urea at early fermentation intervals (2 and 4h) could be a lag phase-phenomenon related to a shortage of required time before normal microbial activity began in the in vitro system. Apparent microbial nitrogen utilization per unit of urea was greater as T increased from 2 to 8 h. At 8 and 10 h AMNU were very similar, probably due to a shortage of nutrients or a toxic effect of fermentation end-products. Overall fermentation time resulted in a curvilinear effect on AMNU across all concentrations of U, with a plateau at fermentation time greater than 6 or 8 h.

Results indicate that under condition of this experiment K decreases AMNU but the decrease does not represent more than 7 percent of the response obtained at zero K concentration. However, microbial response to T suggests the possibility

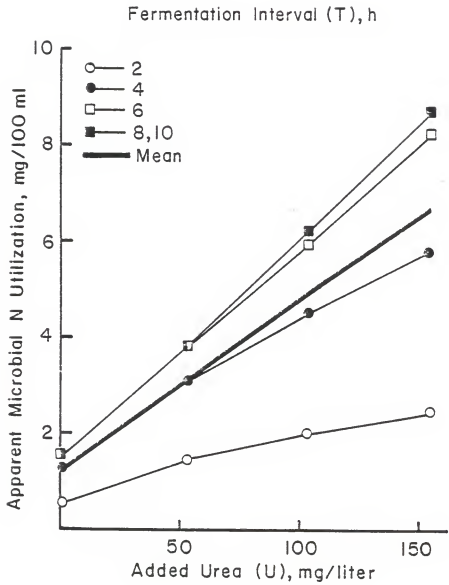

Figure 5. Interactive effect of U and T on apparent microbial N utilization (Experiment 1).

of a shortage of nutrient(s) during fermentation since a plateau response was obtained after 6 h fermentation interval (Figure 4). Consequently, it was decided to study greater concentrations of K and U under similar experimental conditions.

Experiment 2

Concentrations of K and NH_3-N in ruminal fluids collected on different days varied from 1.15 to 1.40 g/liter and 4.9 to 15.0 mg/100 ml, respectively. Ruminal fluid pH was between 6.1 and 6.5. Concentrations of K were quite similar to those in Experiment 1, but mean NH_3-N concentration and pH were higher. During this part of the study, ruminal contents of the donor cow were more liquid than during Experiment 1. Apparently, this was due to lower feed intake and probably higher water intake resulting from higher environmental temperatures (Collier et al., 1982). Concentrations of K and NH_3-N of fermentation fluids (ruminal fluid plus buffer, 1:4) varied from .23 to .29 g/liter and .98 to 3.00 mg/100 ml, respectively.

Means of main effects of R, K, U and T on AMNU are in Table 5, and analysis of variance is in Table 6. All main effects and two and three-way interactions were significant ($P<.001$). Based on same considerations for R as in Experiment 1, three-way interactions involving R were not considered in the biological interpretation of results. Although the K*U*T interaction was significant

TABLE 5. MEANS OF MAIN EFFECTS OF R, K, U AND T ON APPARENT MICROBIAL NITROGEN UTILIZATION (EXPERIMENT 2)

Independent Variable	AMNU[a]	Independent Variable	AMNU[a]
Ruminal Fluid (R)		Added Potassium (K) g/liter	
1	7.68	0	7.48
2	4.44	1.51	7.25
3	6.68	3.02	5.98
4	6.48	4.53	4.56
Standard Error	.090		.090
Urea (U) mg/liter		Fermentation Interval (T), h	
0	1.27	2	1.85
143	5.34	4	4.00
286	8.36	6	6.88
429	10.30	8	8.90
		10	9.97
Standard Error	.090		.101

[a]Apparent microbial N utilization, mg/100 ml of fermentation fluid.

TABLE 6. ANALYSIS OF VARIANCE (EXPERIMENT 2)[a]

Source	d.f.	Mean Squares
Ruminal fluid (R)	3	5.864***
Added K concentration (K)	3	5.779***
Urea concentration (U)	3	49.567***
Fermentation interval (T)	4	29.200***
R*K	9	.415***
R*U	9	1.259***
R*T	12	.513***
K*U	9	.840***
K*T	12	.204***
U*T	12	3.807***
R*K*U	27	.066***
R*K*T	36	.075***
R*U*T	36	.097***
K*U*T	27	.085***
Residual	108	.026

[a]For apparent microbial N utilization, mg/100 ml.

***(P<.001)

(P<.001), direction of the effect of each individual variable on AMNU was similar across combined effects of the other two variables. Thus, only two-way interactions and main effects are discussed.

Pooled means for effect of day of ruminal fluid collection on AMNU varied between 4.44 and 7.68 mg/100 ml of fermentation fluid, a greater range than observed in Experiment 1. Although two-way interactions of R with K, U and T were significant (P<.001), trends of individual effects of K, U and T across and within different ruminal fluids were similar and in the same direction. Potassium addition had a negative effect on AMNU on each day a fermentation was conducted. There was an interaction R*K (P<.001). Means pooled across R, U and T) of AMNU decreased from 7.48 to 4.56 mg/100 ml as K concentration increased from 0 to 4.53 g/liter. However, on most fermentation days, AMNU was similar at 0 and 1.51 g K/liter. A similar effect was observed in Experiment 1 for concentrations of K between 0 and 1.74 g/liter. Response to U on AMNU varied with R (R*U, P<.001), but the general trend was similar. As U concentrations increased, AMNU increased (Table 5). Pooled least squares means for effects of U on AMNU differed between 1.27 to 10.3 mg/100 ml. This response to U was greater than obtained in Experiment 1, probably due both to fermentation fluid used and to higher concentrations of U. Effect of T on AMNU was different on

different fermentation days (R*T, P<.001), but the direction of the effect was similar within each R. In general, AMNU increased from 1.85 to 9.97 mg/100 ml as T increased from 2 to 10 h (Table 5).

The K*U interaction is illustrated in Figure 6. In general, AMNU decreased as K increased. However, response varied little at 0 and 1.51 g K/liter, but decreased much more rapidly at higher K concentrations except at zero U concentration. Likely this lack of effect with zero U concentration was associated with low availability of N for microbial growth. Curvilinear effect of K on AMNU was similar across the three highest U concentrations. Although the quadratic*linear term was significant (P<.008), 95 percent of variation associated with this interaction was explained by the linear*linear term (P<.001). Apparent microbial nitrogen utilization per g K (slope of linear regression of AMNU on K over U) was greater as concentrations of U increased. The extent of utilization changed from 0 to -1.262 mg/g K as U concentration increased from 0 to 429 mg/100 ml. At each K concentration AMNU increased as U concentration increased. However, extent of AMNU per unit of U decreased from .026 to .014 mg/mg urea as K concentration increased from 0 to 4.54 g/liter.

Interaction of K*T is presented in Figure 7. In general, effect of K on AMNU was similar at different fermentation intervals. Most of the variability (70 percent) associated

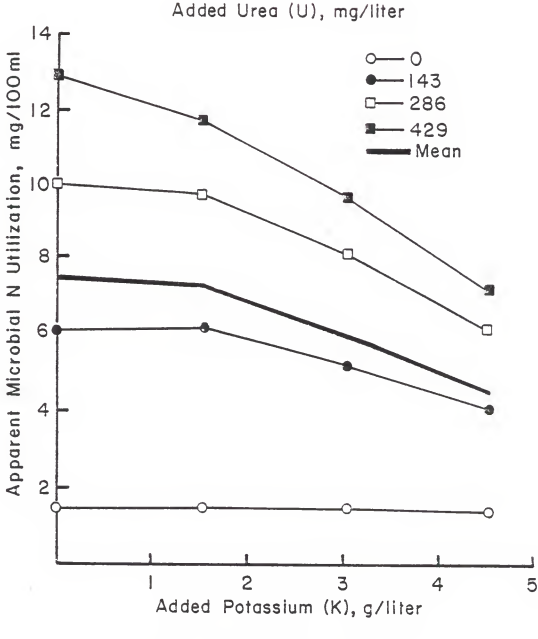

Figure 6. Interactive effect of K and U on apparent microbial N utilization (Experiment 2).

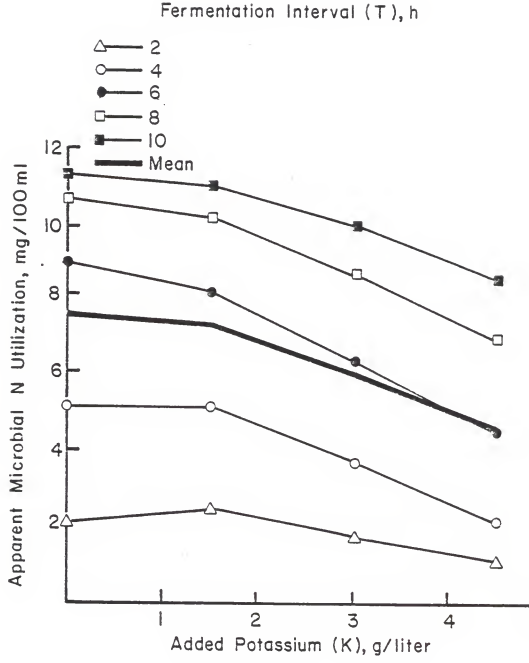

Figure 7. Interactive effect of K and T on apparent microbial N utilization (Experiment 2).

with this interaction was produced by the linear*quadratic term (P<.001). The extent to which K concentration affected AMNU increased up to 6 h fermentation intervals, then decreased (.258, .688, .987, .813 and .607 mg/g K, respectively). This response of microorganisms to K at greater fermentation intervals was similar to those observed in Experiment 1, and may be associated to a gradual adaptation of microbes to high concentrations of K. Anagnostopoulos and Dhavises (1982) showed that E. coli adapted to high osmolality as a result of NaCl addition to the medium. At each concentration of K, AMNU increased as T increased. Microbial response to T was almost equal at 0 and 1.51 g K/liter, but decreased as K increased from 1.5 to 4.53 g/liter.

In the interaction U*T (Figure 8), U increased AMNU at each T. Microbial response to U appears to be curvilinear, specially at 2, 4 and 6 h fermentation times. However, the linear*linear term accounted for 97 percent of variability associated with this interaction. The AMNU rates per unit of U increased from .001 to .007 mg/mg urea as T increased from 2 to 10 h. Effect of T on AMNU increased at each U concentration, and AMNU rates per unit of T were greater as U increased from 0 to 429 mg/100 ml (.032 to .381 mg/h, respectively). This suggests AMNU response was related directly to availability of nitrogen for microbial growth.

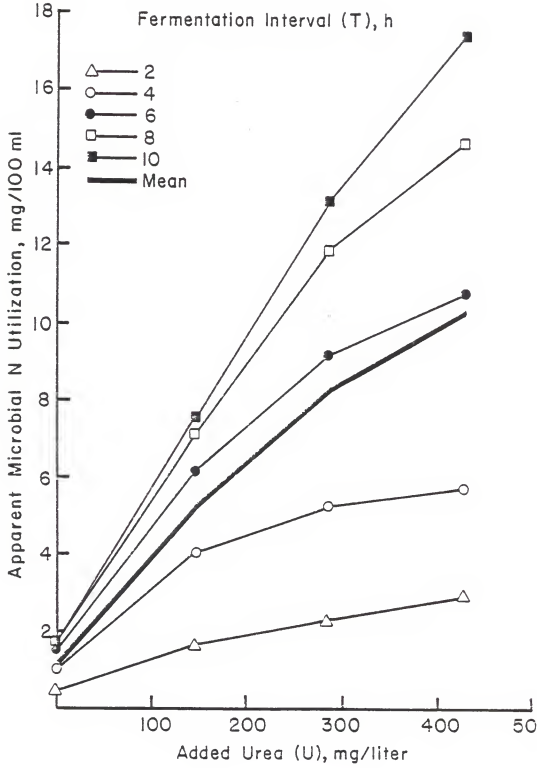

Figure 8. Interactive effect of U and T on apparent microbial N utilization (Experiment 2).

Differences in ruminal fluids between experiments were expected since feed intake and environmental conditions differed. Ruminal pH was an indication of this fact. However, comparing results of the two experiments, the general responses to K, U and T on AMNU were not dramatically changed by ruminal fluids.

In both experiments, over K concentrations tested, AMNU decreased (Table 3 and 5). This indicates that initial concentrations of K in fermentation fluids (ruminal fluid plus buffer, 1:4, without added K treatments) were .22 to .29 g/liter, which were adequate for microbial growth. Mackie and Therion (1984) indicated that growth rates of various pure cultures of ruminal organisms increased rapidly at K concentrations between .002 and .025 g/liter, and optimum growth occurred at concentrations between .196 and 3.91 g K/liter. Different studies (Hubbert et al., 1958; Bryant et al., 1959) indicated maximum growth or bacterial activity occurred with concentrations of K between .1 and 2 g/liter, although Caldwell et al. (1973) did not obtain a negative effect on Bacteroides amylophylus with concentrations up to 3.0 g/liter. However, results of the present study showed a general decrease in AMNU as K concentration increased over a range of 0 to 4.54 g/liter, although a slight decrease between 0 and about 1.50 g K/liter occurred. Microbial response to K or any nutrient depends on microbial and chemical composition of the culture. Caldwell and Hudson

(1974) found different responses to Na, K and other minerals among different species of ruminal bacteria.

Mackie and Therion (1984) reported that optimal microbial growth occurred at Na concentrations between 1.60 and 3.90 g/liter, and was inhibited severely above 9.20 g/liter. In the present study Na concentrations in fermentation fluids varied from 4.8 to 5.0 g/liter, which could reduce microbial growth. Also, Bryant et al. (1959) reported a Na*K interaction on microbial growth, where at lower concentrations of Na higher concentrations of K were necessary for optimum microbial growth. Thus, possibly differences found in optimum K concentration for microbial growth between this study and those reported by Hubbert et al. (1958), Bryant et al. (1959) and Mackie and Therion (1984) may be attributed to differences in Na concentrations.

Observed deleterious effect of K on AMNU may be related to changes in the osmolality of the medium (Durand and Kowashima, 1980). At high osmolalities, microbial cells are unable to maintain intracellular osmolality, since this would involve impermeability to water or continued active excretion of solutes (Mackie and Therion, 1984). They found that at osmolalities higher than 400 to 500 mOsm/kg growth rates (per hour) of Selenomonas ruminantium decreased either with Na or K as the osmotic agent. Results of the present study indicated a decrease in AMNU rate (per hour) as K concentration increased. In addition to the effect on

osmolality, Mackie and Therion (1984) demonstrated that K and Na have specific chemical effects on microorganisms.

The fact that in our study extent of AMNU per unit of K decreased as fermentation time increased from 2 to 6 h and then decreased at 8 and 10 h suggests the possibility of a partial adaptation to K concentration. Anagnostopoulos and Dhavises (1982) and Mackie and Therion (1984) showed bacteria have an adaptative capacity to high osmolalities. A possible mechanism involves intracellular generation of organic molecules such as amino acids and carbohydrates, which act both as osmoregulators and protectors of enzyme activity (Brown, 1976; Roller and Anagnostopoulos, 1982).

The extent to which K affected AMNU increased as the concentration of U increased (K*U, $P<.001$). Apparently this effect is not related to a direct effect of ammonia on microbial cells, since Satter and Slyter (1974) did not observe inhibitory effects on microbial growth with ammonia concentrations as high as 800 mg/liter. Furthermore, in our experiment the high concentration of urea (429 mg/liter) did not decrease AMNU. In both experiments, the effect of U on AMNU was almost linear, but urea utilization was decreased by K. This agrees with observations of Khan et al. (1969) that high concentrations of K affected microbial utilization of urea. This effect may be related to osmotic and chemical effect of K on microbial cells.

Potassium chloride was used as source of K in our studies, and changes in AMNU were attributed to K without any consideration of Cl. Apparently, bacteria have variable requirements for Cl (Caldwell et al., 1973). Reviews regarding influence of minerals in ruminal microbes did not mention Cl as an important or detrimental influence on bacteria. Different studies (Hubbert et al., 1958; Lester, 1958; Bryant et al., 1959; Khan et al., 1969) regarding effect of Na and K and using chloride salts did not associate microbial response to Cl. It seems that high concentrations of Cl do not have deleterious effects on bacteria.

CHAPTER 4

EFFECTS OF MINERAL SALTS ON RUMINAL KINETICS, UREA-NITROGEN UTILIZATION, MICROBIAL PROTEIN SYNTHESIS AND OTHER DIGESTIVE PARAMETERS

Introduction

Different dietary and environmental factors affect ruminal kinetics, e.g., level of intake (Minson, 1966; Evans, 1981a), dietary roughage content (Bauman et al., 1971), inorganic salts (Thomson et al., 1978; Rogers and Davis, 1982a), and environmental temperature (Kennedy et al., 1976). Feeding inorganic mineral salts may be a practical means to manipulate these ruminal factors since they increase osmotic pressure, which plays a role in regulating digesta turnover rate, especially dilution rate, by changing water flux (Warner and Stacy, 1972). This positive effect of inorganic salts on dilution rate has been corroborated administering artificial saliva (Harrison et al., 1975; Thomson et al., 1975, 1978), NaCl (Potter et al., 1972; Rogers and Davis, 1982b) and $NaHCO_3$ (Rogers and Davis, 1982a; Rogers et al., 1982). Rogers et al., (1982) obtained 20 percent increase in ruminal dilution rate with dairy cows when a level of NaCl as low as 2 percent of diet dry matter was fed.

Increased ruminal turnover may be of benefit to the host. This would be most valuable in terms of microbial growth efficienty, improvement in utilization of dietary and recycled nonprotein nitrogen and reduction in fermentation losses for feeds that can be digested more efficiently in the intestine (Bull et al., 1979).

In vitro (Isaacson et al., 1975; Harrison and McAllan, 1980) and in vivo studies (Harrison et al., 1975; Walker et al., 1975; Cole et al., 1976; Prigge et al., 1978) demonstrated that accelerating liquid turnover rate increased microbial yield and microbial efficiency. Apparently, this effect is associated with a reduction in energetic cost for maintenance of microbial population and lysis and predaction of bacteria by protozoa (Isaacson et al., 1978; Harrison and McAllan, 1980).

Some in vitro evidences (Isaacson et al., 1975; Koenig, 1980) suggested that higher turnover rates increased microbial utilization of urea. However, these experiments were not designed to study this effect. Bull et al. (1979) indicated that ruminal turnover probably improves the success in using nonprotein nitrogen, but quantitative data are not available.

Objectives of the present study were to study effects of changing ruminal kinetics with dietary sodium and potassium salts on microbial protein synthesis using urea as

sole source of supplementary nitrogen, ruminal parameters, digestibility and lactational performance.

Materials and Methods

Treatments and Animals

Effects of two dietary mineral treatments were studied using a single reversal design. Control treatment contained .6 percent potassium chloride and no added sodium chloride and high-mineral treatment had 2.13 percent potassium chloride, and 1.95 percent added sodium chloride; potassium and sodium contents of the diets were 1.03, .19 and 1.86, .58 percent (dry basis) for control and high-mineral diets, respectively (Table 7 and 10). Control treatment was formulated to approximate NRC (1978) requirements and high-mineral treatment was based on previous results of lactation studies (Beede et al., 1983a).

Six midlactation Holstein cows fitted with flexible cannulae in the rumen and abomasum were used. Abomasal T-cannulae were made of sylastic tubing (Dow Corning Corp., Midland, Michigan). Dimension of T-cannulae were: horizontal length, 15 cm; vertical length, 20 cm; and inside and outside diameters of exit tube, .62 and .68 cm, respectively. Cannula was installed as close to the abomasal-omasal orifice as possible (about 2 to 5 cm). T-cannulae was exteriorized on the ventral aspect of the abdomen with the exit tubing residing in a vertical posture.

TABLE 7. COMPOSITION OF DIETS

Ingredient	DIET, % (dry basis)		
	Pre-adaptation	Control	High mineral
Ground yellow corn	54.30	55.60	53.00
Cottonseed hulls	40.00	40.00	40.00
Urea	1.50	1.50	1.50
Dicalcium phosphate	1.30	1.30	1.30
Limestone	.33	.33	.33
KCl	1.37	.60	2.13
NaCl	.52	0.00	1.05
MgO	.20	.18	.22
Trace mineral salt and vitamins	.45	.45	.45

Cows were fitted with the ruminal and abomasal cannulae between 3 to 6 mo and 15 days before beginning the experiment, respectively.

Experimental Diets

Three diets (Table 7) were formulated according to several criteria. Feed ingredients chosen contained little or no cytosine or adenine. Cottonseed hulls and urea contents of diets were the same. Mineral and vitamin contents were formulated to be the same except for sodium, potassium and chloride. Pre-adaptation diet was prepared by mixing equal parts of control and high-mineral diets.

Digestion and Microbial Markers

Ytterbium-marked fecal fiber was used to trace the solid phase. It was prepared similarly to the procedure of Vargas and Prigge (1982). Washed fecal material from animals consuming the pre-trial diet was used to prepare the marked fiber. Chromium EDTA complex, marker of the ruminal liquid phase, was prepared using the procedure described by Binnerts et al. (1968). Permanganate lignin was used for measuring ruminal digesta outflow (Cole et al., 1976). Dietary source of lignin was cottonseed hulls since it provided an adequate level of lignin in diets, and apparently this lignin was undigestible (Muntifering, 1982).

Cytosine and adenine were used as markers to estimate microbial protein according to the method of Koenig (1980). Laboratory analyses indicated no detectable cytosine in cottonseed hulls or yellow corn grain, and no adenine in cottonseed hulls. Concentration of adenine in yellow corn grain was 59.8 μg/100 g dry matter.

Feeding and Management of Animals

Cows were fed once daily at 0900 h. Feed intake was ad libitum and adjusted every day so that an excess of 5 to 10 percent was refused. Feeding and milking (twice daily) was performed in individual tie-stalls. Cows were moved to an outside corral for 24 h at the beginning of each adaptation period without affecting their feeding. Diets were introduced on the first day of each dietary treatment adaptation period. Initially, all cows received the pre-adaptation diet for 15 days. Then the experiment was divided in two periods, A and B. In each period, cows were fed respective dietary treatments for 20 days (three cows on control diet and three cows on high-mineral diet). Each period included 10 days of adaptation to diet and 10 days of sampling. Sequence was repeated in the second period.

Analyses, Measurements and Calculations

Ruminal dilution rate and volume. A single dose of 1.5 liters of CrEDTA solution (4.33 g of chromium) was introduced into different parts of the rumen at 0800 h using

a funnel and tube. Ruminal fluid was sampled from five different sites of the rumen by manual placement of a 50 ml plastic tube. At the same time, six to eight small solid samples were collected from different parts of the rumen (200 to 300 g of fresh material in total). Both liquid and solid samples were strained through a double layer of cheesecloth. Sampling frequency was every 3 h over 26 h, except for the first sample which was taken 2 h after dosing.

Approximately 25 ml of ruminal fluid was treated with a saturated solution of $HgCl_2$ (.25 ml) and centrifuged at 3000 x g for 10 min. Supernatant was stored in a freezer (-10°C). For the analysis of chromium using atomic absorption spectrophotometry (Model 5000, Perkin-Elmer, Norwalk, CT), supernatant samples were deproteinated with approximately 30 mg of sulfosalycylic acid/ml and centrifuged at 3000 x g for 10 min. Standards for analyses were prepared in a matrix of ruminal fluid collected from each cow before dosing with marker. For the matrix a composite sample from all cows was prepared per period and treated in the same way as individual samples.

Rate of disappearance of Cr in the rumen, which represents ruminal liquid turnover rate, was estimated using the following equation (Faichney, 1975)

$$\ln Y = A - Kt \qquad (1)$$

where.

ln Y = natural logarithm of Cr concentration, mg/liter

t = accumulative time after dosing marker, h

A = intercept at t = 0

K = rate of disappearance of Cr from the rumen per hour

Ruminal dilution rate (D), expressed as percent/h was obtained by multiplying K by 100. Ruminal concentration of Cr (Co) at t = 0 (mg/liter) was obtained using the antilogarithm of A.

Ruminal volume (RV, liters) was calculated using the following equation

$$RV = \frac{\text{Cr dose, mg}}{Co} \qquad (2)$$

Ruminal solid turnover rate and retention time using the rate of disappearance of Yb in the rumen. A single dose of 1.0 kg of ytterbium-marked fiber (14.5 g of ytterbium) was distributed in different parts of rumen immediately before dosing the CrEDTA. Samples were collected every 3 h during the first day, and every 6 h on days 2, 3 and 4 post dosing. They were obtained following the procedure indicated for collection of samples to determine ruminal dilution rates. Solid material remaining after straining the ruminal sample through cheesecloth was used for the Yb

analyses. After the first day, sampling procedure did not include collection of liquid samples, only solid samples.

Approximately 200 g of strained solid material was dried at 65°C for 48 h and then ground in a Wiley mill (2 mm). Ytterbium was extracted using the procedure of Hart and Polan (1984) and analyzed using atomic absorption spectrophotometry (Model 5000, Perkin-Elmer, Norwalk, CT). One composite sample per period was prepared with ruminal solid material taken from each cow before dosing the marker and used to determine background in the analyses of Yb.

Rate of disappearance of Yb in the rumen, which represents ruminal turnover rate of the solid phase, (STR, percent/h), was estimated using a similar mathematical relationship described for Cr disappearance.

Solids retention time (SRT, h) was estimated as the inverse of solids turnover rate (Faichney, 1975)

$$SRT = \frac{1}{STR/100} \tag{3}$$

Ruminal solid turnover rate and retention time using the rate of appearance of Yb in feces. Starting 8 h after dosing the marker, fecal grab samples were taken every 3 h during the first day, every 6 h on days 2, 3 and 4, and every 12 h on days 5 and 6. Approximately 100 g of fresh sample was dried at 65°C for 48 h and ground in a Wiley mill (2 mm). As in the case of ruminal Yb, fecal material

was taken before dosing with the marker and used for background correction in the analysis of Yb.

A two-compartment age-dependent model (Ellis et al., 1979) was used to describe appearance of Yb in the feces and to estimate the STR. Mathematical model was as follows

$$Y = \left[Co\, e^{-K_1(t-T)}\right]\left[\frac{K_1^2(t-T)}{K_2-K_1} - \frac{K_1^2}{(K_2-K_1)^2}\right] + \left[e^{-K_2(t-T)}\left(\frac{K_1}{K_2-K_1}\right)^2\right] \quad (4)$$

where

Y = Yb concentration in feces, mg/kg dry matter

t = accumulative time after dosing the marker, h

T = time delay, h

Co = initial concentration of Yb in the time-independent compartment, mg/kg

K_1 = time-dependent turnover rate per hour

K_2 = time-independent turnover rate per hour

Time delay represents time between dosing of marker into the rumen and its first detectable appearance in feces. Time-dependent turnover represents the mixing rate of the feed and (or) Yb-marked fecal fiber in the rumen and time-independent turnover represents the solids turnover rate in the rumen expressed as percent/h. Data were analyzed by non-linear regression procedures using the Marquardt method (SAS Institute, Inc., 1982).

Solids retention time was estimated using the same mathematical relationship indicated for ruminal Yb disappearance.

Ruminal liquid outflow. Ruminal liquid outflow (RLF, liters/day) was estimated as follows (Faichney, 1975)

$$RLF = \frac{24\ (RV)D}{100} \qquad (5)$$

where

RV = ruminal liquid volume, liters

D = dilution rate, percent/h

24 = hours in a day

Ruminal digesta outflow. Samples of digesta from the abomasum were taken two times per day for 9 days. A 50 ml syringe fitted with a 30 cm length of flexible tubing was used for intraabomasal mixing of contents and sampling. However, in many cases samples were taken directly since digesta flowed after mixing abomasal contents. Subjective assessment suggested that digesta flow was stimulated by mixing and sampling. About 100 to 150 ml of abomasal contents were taken each time to assure an adequate and heterogeneous sample. In most cases each quantity of sample needed was taken within 5 min. Samples were collected at different times each day similar to procedure outlined by Theurer et al. (1981) and treated with a saturated solution of $HgCl_2$ (1 ml per 100 ml of sample). A composite sample

was prepared every day from each cow and stored in a freezer (-10°C) for laboratory analyses. Then a composite sample per cow-period was prepared. A subsample was dried at 65°C for 24 h and ground in a Wiley mill (2 mm). Lignin analyses were performed using the permanganate procedure (AOAC, 1975).

The ruminal digesta outflow (RDF) was estimated by the following equation (Faichney, 1975)

$$RDF = \frac{LI}{LCD} \tag{6}$$

where

RDF = ruminal digesta outflow, kg wet material/day

LI = lignin intake, kg/day

LCD = lignin content of abomasal digesta, kg/kg wet material

Microbial N outflow. A subsample from the abomasal composite sample prepared for measuring ruminal digesta flow was taken to estimate microbial nitrogen content of digesta. This measurement was estimated using ratio relationship of both cytosine and adenine with nitrogen. Samples for the analysis of cytosine and adenine were prepared generally according to the procedure of Koenig (1980) using high pressure liquid chromatography (HPLC) procedures. About 5 to 10 g of sample was dried (90°C for 24 h) and then hydrolyzed with 2.5 ml of 70 percent perchloric acid for 1 h at 90°C in a water bath with continuous swirling. Samples were

diluted to 250 ml and filtered through a .45 micron millipore filter before analysis by HPLC. For the separation of nucleotides a cation exchange column (Partisil-10 SCX, Reeve Angel and Co., Clifton, NJ) was used. This was preceded by a small guard column packed with a cation exchange resin (Altex Scientific, Inc., Berkeley, CA) used to remove extraneous materials. Samples were eluted from the column with a .2 M $NH_4H_2PO_4$ buffer adjusted to a pH 3.2 with concentrated HCl.

Microbial nitrogen content of digesta (AMNC), using cytosine as microbial marker, was calculated using the following equation

$$\text{AMNC} = \frac{\text{Abomasal cytosine content}}{\text{Microbial cytosine-to-N ratio}} \qquad (7)$$

where AMNC value was expressed as g/kg wet abomasal digesta, abomasal cytosine content in μmol/kg wet abomasal digesta, and microbial cytosine-to-N ratio in μmol/g. Adenine also was used as a similar microbial marker; AMNC also was estimated using adenine with the same mathematical relationship as with cytosine. Estimates of AMNC for each cow-period were calculated using its individual cytosine (or adenine)-to-N ratio.

Microbial cytosine (or adenine)-to-N ratio was determined for each cow using ruminal material taken twice daily before starting the sampling period and on the last day of

each period. Approximately 300 ml of ruminal fluid per cow was collected twice a day at 8-h intervals, strained through a double layer of cheesecloth and treated with a saturated solution of $HgCl_2$ (1 ml per 100 ml of sample). Samples were processed by differential centrifugation according to the procedure of Koenig (1980) to isolate the microbial material. Strained ruminal fluid was centrifuged at 500 x g for 10 min. Then supernatant was centrifuged at 30,000 x g for 25 min. Solid material remaining, which represented microbial material, was washed with 15 ml of deionized water and centrifuged again at 30,000 x g for 25 min. One composite sample of microbial material was prepared per cow-period and stored in a freezer at -10°C. Cytosine and adenine were analyzed using the procedure of Koenig (1980) and nitrogen was analyzed using the procedure of Technicon Industrial System (1978). For estimation of microbial nucleotide-to-N ratios, microbial contents of cytosine, adenine and nitrogen were expressed on a dry basis.

Calculations of ruminal microbial nitrogen outflow (RMNO, g/day) were made as follows

$$RMNO = AMNC \times RDF \qquad (8)$$

Estimates of RMNO were calculated separately using each cytosine and adenine as microbial markers, with lignin as the flow marker.

Variability of cytosine and adenine contents. Variability of cytosine and adenine concentrations in digesta was studied using composite samples taken daily during the last 5 days of abomasal sampling for the estimation of RMNO. Samples were prepared and analyzed according to the procedure of Koenig (1980).

Ruminal pH, ammonia, volatile fatty acids and osmolality. Liquid-phase ruminal samples were obtained using the procedure indicated for Cr. They were collected 4 h after feeding during the last 6 days of each sampling period. The pH was measured immediately after collection. Approximately 20 ml of ruminal fluid were treated with $HgCl_2$ and stored in a freezer at -10°C in polyethylene tubes for the analysis of NH_3-N and volatile fatty acids. Approximately 10 ml of ruminal fluid were stored in the same condition for analysis of osmolality. Composite samples per cow-period were prepared for the analysis of NH_3-N, volatile fatty acids and osmolality. For the analysis of NH_3-N and volatile fatty acids, samples were deproteinated using the procedure indicated for Cr samples. Analysis of NH_3-N was carried out according to the procedure of Technicon Industrial System (1978) and volatile fatty acids were analyzed using gas chromatography (Sepulco, Inc., 1975). Osmolality was determined by freezing-point depression (Potter et al., 1972).

Digestibility of organic matter, nitrogen and acid-detergent fiber, lignin recovery and nitrogen balance. Schedule for measuring feed intake, and fecal and urinary output, and for sampling feed, orts, feces and urine are indicated in Table 8. Samples were taken as a constant proportion of the total dry matter (1 percent for feed, 5 percent for orts and 2 percent for feces). In the case of urine, .5 percent volume was taken. Cows were fitted with indwelling urethral catheters (Foley, 22 French, 75 ml ovoid, C.R. Bard, Inc., Murry Hill, NJ), to allow separation of urine from feces throughout the collection period. Feed and orts samples were stored at $4^{o}C$, and wet fecal samples were stored in a freezer ($-10^{o}C$). Samples were then dried at $65^{o}C$ for 48 h and allowed to equilibrate with atmospheric conditions for 2 days.

Composite samples per cow-period were prepared for different analyses. Organic matter, acid-detergent fiber and lignin were analyzed using methods of AOAC (1975), and nitrogen was analyzed using the method of Technicon Industrial System (1978). Feed intake, apparent digestion coefficients of nutrients and lignin recovery were estimated from total collection of feces and calculated using equations described by Schneider and Flatt (1975). Nitrogen balance was calculated by difference between nitrogen intake and excretion in feces and urine.

TABLE 8. COLLECTION SCHEDULE FOR DIGESTIBILITY AND NITROGEN TRIAL

Sampling day	Weight				Sample			
	Feed	Orts	Feces	Urine	Feed	Orts	Feces	Urine
1	x	No	No	No	x	No	No	No
2	x	x	No	x	x	x	No	x
3	x	x	x	x	x	x	x	x
4	x	x	x	x	x	x	x	x
5	x	x	x	x	x	x	x	x
6	x	x	x	x	x	x	x	x
7	x	x	x	x	x	x	x	x
8	No	x	x	x	No	x	x	x
9	No	No	x	No	No	No	x	No

Water Intake. Water intake of individual cows was measured daily during each sampling period using in-line flow meters. Readings were taken at 1000 h each day.

Ruminal digestion of organic matter. Organic matter (OM) was determined in same dried samples of digesta prepared for the analysis of lignin. Apparent OM disappearing from rumen (ROMD) was estimated using lignin as the marker of digesta. Calculation was made as follows (Muntifering, 1982)

$$\text{ROMD, \%} = 100 - 100 \frac{\text{(Lignin content in OM consumed), g/kg}}{\text{(Lignin content in digesta OM), g/kg}} \quad (9)$$

True OM fermented in the rumen (ROMF), which considers microbial OM in the digesta as part of the OM fermented in the rumen, was estimated using a proportion of 1(N):9(OM) for microbial cells (McAllan and Smith, 1977)

$$\text{ROMF \%} = 100 \frac{\text{(OMI) (ROMD/100)} + 9\text{(RMNO)}}{\text{OMI}} \quad (10)$$

where OMI represents OM intake, kg/day.

Efficiency of microbial nitrogen production. Efficiency of microbial nitrogen production was estimated with respect to both ROMD and ROMF (Czerkawaski, 1978) as follows

$$\text{EMNPa} = \frac{\text{RMNO}}{\text{(OMI) (ROMD/100)}} \quad (11)$$

$$EMNPT = \frac{RMNO}{(OMI)(ROMF/100} \quad (12)$$

where

EMNPa = Apparent efficiency of microbial N production, g/kg OM disappearing within the rumen

EMNPt = True efficiency of microbial N production, g/kg OM truly fermented within the rumen

Milk production and composition. Individual milk production of each cow was measured during the first 7 days of each sampling period. Milk samples were taken during the last 2 days of each sampling period for analyses of protein, fat and somatic cell contents (D.H.I.A., Raleigh, NC).

Statistical Analysis

Statistical analysis was by analysis of variance using general linear model procedures of SAS (SAS Institute, Inc., 1982). Model used was

$$Y_{ijk} = U + P_i + C_j + T_k + E_{ijk}$$

where

U = Overall mean

P_i = Fixed effect of ith period

C_j = Random effect of jth period

T_k = Fixed effect of kth treatment

E_{ijk} = Residual effect

Results and Discussion

Laboratory analyses of diets are in Table 9. Contents of sodium (Na) for both diets were very close to those formulated, but potassium (K) concentrations were slightly higher.

Least squares means for dry matter (DM), crude protein (CP), Na, K and water intake are in Table 10. There was no treatment effect on DM intake or crude protein intake ($P>.10$, Table 11). However, with similar contents of Na and K, but a diet based on ground corn, soybean meal and cottonseed hulls, Beede et al. (1983a) found a higher DM intake with a high Na(.7 percent) and K(1.58 percent) diet. In that and the present experiment, DM intakes of diets with Na and K concentrations similar to recommendations of NRC (1978) were about 22 kg DM/cow/day.

Dietary treatment effect on water intake was significant ($P>.01$), but also its interaction with sampling day (T*D, $P<.05$, Table 11). High-mineral treatment increased water intake 29% over control. Different authors (Potter et al., 1972; Rogers et al., 1979; Rogers and Davis, 1982b) obtained similar effects on water intake using high Na diets or intraruminal infusion of Na solutions from the chloride salt. They indicated that this effect was due to an increase in ruminal osmolality produced by added sodium salts.

TABLE 9. LABORATORY ANALYSES OF EXPERIMENTAL DIETS[a]

Component, %	Diet	
	Control	High-Mineral
Organic matter	95.40	92.90
Crude protein	11.40	11.20
Acid detergent fiber	32.70	35.80
Permanganate lignin	5.70	7.20
Sodium	.19	.58
Potassium	1.03	1.86
Calcium	.47	.40
Phosphorus	.46	.37
Magnesium	.24	.34
Sulfur	.12	.11
Estimated TDN	67.00	64.00
Estimated NE_{ℓ}, Mcal/kg	.70	.66

[a]Dry matter basis.

TABLE 10. DIETARY TREATMENT EFFECTS ON DAILY DRY MATTER, CRUDE PROTEIN, SODIUM, POTASSIUM AND WATER INTAKE[a]

Measurement	Treatment		
	Control	High-Mineral	S.E.
Dry matter, kg	22.20	22.20	.29
Crude protein, kg	2.58	2.49	.08
Sodium, g	41.00	128.00	--
Potassium, g	228.00	412.00	--
Water, liter	79.00	101.70	2.00

[a]Least squares means.

TABLE 11. LEAST SQUARES ANALYSIS OF VARIANCE FOR DRY MATTER, CRUDE PROTEIN AND WATER INTAKE

Source	d.f.	Mean Squares		
		Dry Matter	Crude Protein[a]	Water
		---------- kg/day ----------		liter/day
Period (P)	1	63.740^{NS}	.077^{NS}	129.886^{NS}
Treatment (T)	1	.632^{NS}	.026^{NS}	9464.212****
Cow (C)	5	154.344****	.282**	3126.226****
P*T*C[b]	4	16.999****	---	36.200^{NS}
Day (D)	6	4.632^{NS}	---	627.318****
P*D	6	1.863^{NS}	---	367.209****
T*D	6	1.188^{NS}	---	185.882**
C*D	30	2.733^{NS}	---	93.239^{NS}
Residual (R)	21	2.526	.038	61.632

[a]Degrees of freedom for residual = 4

[b]Pooled interactions (P*T + P*C + T*C + P*T*C)

^{NS}(P>.10)

**(P<.05)

****(P<.001)

Means for ruminal kinetic measurements are in Table 12 and analyses of variance are in Table 13. There was no significant (P>.10) treatment effect on dilution rate, although a slight tendency toward a higher dilution rate was observed with the high-mineral treatment. However, ruminal volume was increased 15 percent by high-mineral treatment (P<.01). Ruminal volume correlated positively, with water intake but correlation was not high (r=.-3, P<.01). Probably, part of the effect of high-mineral treatment on ruminal volume was due to an intra-ruminal influx of water since salivary secretion is reduced as ruminal osmolality increases (Warner and Stacy, 1977). In lactating cows, Rogers et al. (1982) obtained a significant increase in dilution rate but not in ruminal volume with addition of 2 percent of NaCl or more to a basal diet. However, in a study with steers (Rogers and Davis, 1982b) dietary NaCl increased both dilution rate and ruminal volume. Dilution rates obtained in this experiment were similar to those reported by Rogers et al. (1982) with cows producing 25 to 30 kg/day, but ruminal volumes in our experiment were about 35 percent greater. This may be related to difference in nature of diets fed; cottonseed hulls plus concentrate in our experiment compared to corn silage plus concentrate in the experiment of Rogers et al. (1982). Harrison et al. (1975) observed that mineral salts increased

TABLE 12. DIETARY TREATMENT EFFECTS ON RUMINAL KINETIC MEASUREMENTS[a]

Measurement	Treatment		
	Control	High-Mineral	S.E.
Dilution rate (D), %/h	12.0	12.4	.17
Volume (RV), liter	62.8	72.4	1.45
Liquid outflow (RLF), liter/day	180.9	207.5	6.00
Solids turnover rate (STR), %/h			
By ruminal Yb disappearance	4.5	4.7	.15
By fecal Yb appearance	3.9	4.1	.23
Solids retention time (SRT), h[b]			
By ruminal Yb disappearance	22.2	21.6	6.70
By fecal Yb appearance	25.6	24.4	4.40
Digesta outflow (RDF), kg/day[c]	170.5	242.8	12.00

[a]Means.

[b]$SRT = \frac{1}{STR/100}$

[c]Digesta as collected contains solids and liquid fractions.

TABLE 13. ANALYSIS OF VARIANCE FOR RUMINAL KINETIC MEASUREMENTS

Source	d.f.	Dilution Rate (D)	Ruminal Volume (RV)	Solid Turnover Rate (STR)		Ruminal Outflow		Ruminal N outflow (RNO)
				R[a]	F[b]	Liquid (RLF)	Digesta (RDF)	
		- %/h --	-- liter --	---------- %/h	----------	- liter/day -	-- kg/day --	--- g/day ---
Period	1	.704*	114.083**	.213[NS]	.141[NS]	63.940[NS]	4844.101*	4904.563*
Treatment (T)	1	.368[NS]	280.333***	.120[NS]	.187[NS]	2233.140**	20213.020***	27917.451***
Cow (C)	5	10.821****	120.945***	1.179**	1.280[NS]	2918.462**	2082.160[NS]	5779.762**
Residual (R)	4	.135	12.658	.137	.932	215.428	857.876	941.698

[a]R = Estimated by ruminal Yb disappearance.

[b]F = Estimated by fecal Yb appearance.

[NS](P>.10)

*(P<.10)

**(P<.05)

***(P<.01)

****(P<.001)

dilution rates only in animals with relatively low dilution rates when fed a control diet. In addition, dilution rates for animals eating same diet appeared to be affected by individual characteristics of animals and by type of diet fed previously (Harrison et al., 1975; Thomson et al., 1975).

Solids turnover rates estimated with both ruminal Yb disappearance and fecal Yb appearance procedures were not affected (P>.10) by the high-mineral treatment (Table 12). Average solids turnover rates obtained with the ruminal disappearance procedure were 15 percent higher than that obtained by fecal-Yb appearance (P<.10). Estimates of solids retention times (h) are simply the inverse of STR. Therefore, relationships of effects of treatments and statistical analysis are the same.

Ruminal liquid outflow and ruminal digesta outflow were increased (P<.05) by the high-mineral treatment (Table 12). For estimating ruminal digesta outflow, lignin was used as the internal marker. Recovery of dietary lignin in feces averaged 100.3 percent (93.7 to 116.6 percent), which agrees with observations of Muntifering (1982). Both outflow measurements were comparable to those reported by Bauman et al. (1971). Different authors (Rogers et al., 1979, 1982) obtained similar effects of mineral salts on ruminal liquid outflow. Apparently, this effect is produced by an increase in ruminal osmolality which increases water intake and ruminal water influx (Warner and Stacy,

1972). With respect to control, high-mineral treatment increased ruminal liquid and digesta outflow by 15 and 42 percent, respectively (Table 12).

The fact that liquid outflow was slightly greater than digesta outflow for control diet may be related to difficulty of estimations of liquid (Cr) and (or) digesta (lignin) phase markers. A similar difference was not noted for high-mineral treatment. There was a positive correlation between water intake and ruminal liquid outflow ($r=.61$, $P<.05$) and water intake and ruminal digesta outflow ($r=.73$, $P<.01$). Also, ruminal digesta outflow correlated with ruminal volume ($r=.81$, $P<.01$). Since dilution rate was not affected by the high-mineral treatment one might expect a correlation between ruminal volume and ruminal liquid outflow. Instead no correlation was found ($r=.38$, $P<.21$).

Means for ruminal non-kinetic measurements are in Table 14, and analyses of variance are in Table 15. Ruminal pH was not affected by high mineral treatment ($P>.10$). Different in vivo studies (Harrison et al., 1975; Rogers et al., 1979; Rogers and Davis, 1982b) have indicated an unconsistent effect of Na salts on pH, which appeared to depend on level and chemical nature of salt administered and type of diet.

Ruminal ammonia concentrations were not changed by high mineral treatment ($P>.10$, Table 14). Values obtained were in range suggested by Huber and Kung (1981) as

TABLE 14. DIETARY TREATMENT EFFECTS ON RUMINAL NON-KINETIC MEASUREMENTS[a]

Measurement	Treatment		
	Control	High-Mineral	S.E.
pH	6.44	6.43	.04
Ammonia, mg/100 ml fluid	20.90	21.20	2.90
Osmolality, mOsm/kg fluid	260.80	284.30	5.10
Sodium, g/liter fluid	2.84	2.30	.09
Potassium, g/liter fluid	1.54	2.01	.04
Total volatile fatty acid, mM	102.70	93.90	4.60
Acetate, molar %	62.80	68.00	.70
Propionate, molar %	28.00	22.90	1.30
Butyrate, molar %	10.30	9.00	.20
Acetate-to-propionate ratio	2.40	3.00	.10

[a]Means

TABLE 15. ANALYSIS OF VARIANCE FOR RUMINAL NON-KINETIC MEASUREMENTS

		Mean Squares									
Source	d.f.	pH	Ammonia	Osmolality	Sodium	Potassium	Total VFA	Acetate	Propionate	Butyrate	Acetate-to-propionate ratio
			mg/100 ml	-mOsm/kg-	g/liter	g/liter	-- mM ---	----------	molar %	----------	
Period (P)	1	.746****	158.413^{NS}	126.750^{NS}	.216*	13.230**	1.080^{NS}	6.308^{NS}	5.333^{NS}	5.468***	1.541^{NS}
Treatment (T)	1	.001^{NS}	.270^{NS}	1656.750**	.880***	68.163****	234.085^{NS}	81.641***	78.030**	5.200***.	138.041***
Cow (C)	5	.628***	45.188^{NS}	563.083^{NS}	.050^{NS}	2.064^{NS}	149.116^{NS}	36.636***	68.388**	1.613**	122.975***
Residual (R)	4	.066	49.202	157.000	.045	1.204	126.347	3.159	10.162	.207	6.308

^{NS}(P>.10)

*(P<.10)

**(P<.05)

***(P<.01)

****(P<.001)

adequate for maximum in vivo microbial growth. Lack of treatment effect on ruminal ammonia concentrations is not clear since high-mineral treatment increased ruminal volume and ruminal liquid and digesta outflow, which implied a lower nutrient concentration in the rumen and a faster passage of nutrients from the rumen, respectively. The fact that ruminal fluid samples for measuring ammonia concentration were taken 4 h after feeding each day could have affected these results since intake may have not followed the same pattern with both diets. However, possibility of a physiological treatment effect on ruminal ammonia absorption or urea-recycling should be considered.

Scott (1960) and Warner and Stacy (1972) found that an increase in ruminal K concentrations affected ruminal permeability and electrical potential. In experiments using ruminal pouch technique, Houpt (1970) found that an increase in osmolality caused both a net flux of water into the pouch and a doubling of net urea transfer into the pouch. Calculations indicated that additional urea transferred to the pouch could be explained as result of solvent drag. Bunn and Matrone (1968) obtained a lower ruminal ammonia concentration for animals fed a basal diet with urea than for those fed with the same diet supplemented with Na and K. An explanation for these results were based on a greater absorption or a higher rate of production of ammonia. However, Jones et al. (1964) found that Na, K and other

cations reduced urease activity. Apparently, diets containing relatively high contents of Na and K may affect ruminal recycling or urea, since at the kidney, ammonia functions to facilitate excretions of anions with a minimal excretion of mineral cations (Bunn and Matrone, 1968). Conversely, when adequate mineral cations are present in the diet, activity of ammonia mechanism is diminished and mineral cations are found in urine (Bunn and Matrone, 1968). Hence, more ammonia may be recycled into rumen.

There was a treatment effect ($P<.01$) on ruminal N outflow. Means were 314 and 398 g/day for control and high-mineral treatment, respectively. With the high-mineral treatment ruminal N outflow (nitrogen concentration in abomasal digesta, g/kg, x digesta outflow, kg/day), was equal to N intake (398 vs 397 g/day). However, with the control treatment a loss of 99 g N/day occurred in the rumen. These results support the idea that high-mineral treatment could affect intraruminal exchange of nitrogen.

Rumen osmolality was increased ($P<.01$) by high-mineral treatment from 260.8 to 284.3 mOsm/kg. This effect of Na and K on ruminal osmolality has been observed in other studies under different experimental conditons (Potter et al., 1972; Harrison et al., 1975; Rogers et al., 1979). However, increased osmolality in our experiment was below that considered by Bergen (1972) as negative to feed intake.

High-mineral treatment reduced ($P<.01$) ruminal Na concentration but increased ($P<.01$) ruminal K concentrations (Table 14). Ruminal Na concentrations were negatively correlated with water intake ($r=-.68$, $P<.01$), ruminal volume ($r=-.83$, $P<.01$) and ruminal digesta outflow ($r=.87$, $P<.01$). Partially, effect of high-mineral treatment on ruminal Na concentrations is explained by the increase in ruminal liquid outflow and ruminal volume. Also, a reduction in salivary Na and an increase in ruminal Na absorption could contribute to this effect since ruminal osmolality reduces salivary secretion (Warner and Stacy, 1977) and increases ruminal Na absorption (Warner and Stacy, 1972). Osmolality also may affect ruminal Na absorption through an effect on activity of key enzymes involved in active transport of the ion across cell membranes (Warner and Stacy, 1972). Also, they found that independent of osmolality an increase in ruminal Na and (or) K concentrations increased ruminal Na absorption.

In the case of K, increase in ruminal liquid outflow and ruminal volume did not override effects of additional intake compared to control treatment, thus ruminal K concentration increased. Ruminal K absorption may not be an important mechanism affecting ruminal K concentration since Warner and Stacy (1972) found that K absorption increased at a slow rate as dietary K supply increased.

Although means for total VFA concentrations in the rumen were higher for control treatments (Table 14), difference was not significant (P>.10). Different studies (Rogers and Davis, 1982a, 1982b) indicated a negative effect of accelerated ruminal liquid kinetics on total VFA concentrations. Molar proportions of volatile fatty acids were affected by high mineral treatment (Table 14). Molar percentage acetate was increased (P<.01) but propionate (P<.05) and butyrate (P<.01) were reduced. These effects on VFA composition agree with those obtained by Harrison et al. (1975), Thomson et al. (1975) and Rogers and Davis (1982a) increasing ruminal liquid turnover of animals fed high concentrate diets. Acetate-to-propionate ratio was increased also (P<.001). Effects on acetate proportion and acetate-to-propionate ratio are important in milk production since milk fat content is positively correlated with molar percentage of ruminal acetate (Davis, 1979).

Microbial cytosine-to-N ratio (CYR) was increased (P<.05) by high-mineral treatment (Table 16). Microbial adenine-to-N (ADR) was greater for high-mineral treatment (Table 16) but increase was not significant (P>.10). Standard error for ADR was six times that of CYR. A treatment effect on these estimates was expected since microbial nucleic acid content, especially RNA, is affected by ruminal kinetics (Bergen and Yokoyama, 1977) but also

TABLE 16. DIETARY TREATMENT EFFECTS ON MICROBIAL MEASUREMENTS[a]

Measurement	Treatment[b]			Treatment[c]		
	Control	High-mineral	S.E.	Control	High-mineral	S.E.
Bacterial cytosine-to-N ratio (CYR), μg/g	252.30	278.00	6.2	---	---	---
Abomasal cytosine, μmol/kg digesta[d]	35.70	33.70	1.7	---	---	---
Bacterial adenine-to-N Ratio (ADR), μg/g	---	---	---	276.30	283.80	38.60
Abomasal adenine, μmol/kg digesta[d]	---	---	---	46.80	43.60	3.20
Abomasal microbial, g/kg digesta	1.32	1.29	.06	1.56	1.31	.06
Ruminal microbial N outflow (RMNO), g/day	226.50	313.50	11.80	266.30	315.60	5.60
Efficiency of microbial N production (EMNPa), g/kg OM disappeared in rumen	20.90	40.30	.80	24.00	39.30	1.40
Efficiency of microbial N production (EMNPt), g/kg OM truly fermented in rumen	17.50	29.30	.70	19.80	29.40	.60

[a]Means.

[b]Using cytosine as microbial marker.

[c]Using adenine as microbial marker.

[d]Using a 9-day composite sample.

by microbial growth rate (Bergen et al., 1982). Ruminal cytosine-to-N ratio was different between periods ($P<.01$, 283.3 and 247.0±6.2 μmol/g for period A and B, respectively) and cows ($P<.01$). Ruminal adenine-to-N ratio was not affected by these factors.

Microbial N concentration of abomasal digesta estimated with both cytosine and adenine decreased with high mineral treatment (Table 16, 17, 18), but decrease was not significant ($P>.10$) with cytosine. Ruminal microbial N outflow (RMNO) was increased ($P<.01$) by high-mineral treatment (Table 16, 17, 18). Adenine gave greater values for RMNO than cytosine but difference was not significant ($P>.10$). In addition to the high variability found for bacterial adenine-to-N ratio, yellow ground corn used to prepare diets contained a small amount of adenine, which if escaping ruminal degradation would overestimate microbial N outflow by 20 to 25 g/day. Koenig (1980) found better estimates of microbial N with cytosine than with adenine primarily because there was less variability. He also found that soluble cytosine was degraded rapidly by ruminal microorganisms and no soluble cytosine was found in abomasal digesta. Conversely, soluble adenine was found in abomasal digesta.

Ruminal microbial N outflow estimated using cytosine correlated positively with water intake ($r=.77$, $P<.01$), ruminal liquid outflow ($r=.49$, $P<.10$), ruminal digesta flow

TABLE 17. ANALYSIS OF VARIANCE FOR MICROBIAL MEASUREMENTS USING CYTOSINE AS MICROBIAL MARKER.

Source	d.f.	Mean Squares					
		Cytosine-to-N ratio (ADR)	Abomasal cytosine	Abomasal micr. N	Ruminal micr. N outflow (RMNO)	Efficiency micr. N production (EMNPa)[a]	Efficiency micr. N production (EMNPt)[b]
		--- µg/g ---	µmol/kg digesta	--g/kg-digesta	--- g/day ---		
Period (P)	1	3945.813***	3.000^{NS}	.178**	441.653^{NS}	1.470^{NS}	.701^{NS}
Treatment (T)	1	1991.763**	11.213^{NS}	.002^{NS}	22689.603***	1125.203****	421.267****
Cow (C)	5	3304.784***	18.512^{NS}	.048^{NS}	1510.760^{NS}	38.828**	13.317*
Residual (R)	4	232.943	17.207	.020	830.643	4.202	2.824

[a] g/kg OM disappearance in rumen.

[b] g/kg OM truly fermented in rumen.

^{NS} (P>.10)

* (P<.10)

** (P<.05)

*** (P<.01)

**** (P<.001)

TABLE 18. ANALYSIS OF VARIANCE FOR MICROBIAL MEASUREMENTS USING ADENINE AS MICROBIAL MARKER

Source	d.f.	Mean Squares					
		Adenine-to-N ratio (ADR)	Abomasal adenine	Abomasal micr. N	Ruminal micr. N outflow (RMNO)	Efficiency micr. N production (EMNP)[a]	Efficiency micr. N production (EMNPE)[b]
		µg/g	µmol/kg digesta	g/kg digesta	--- g/day ---		
Period (P)	1	8899.851^{NS}	96.901^{NS}	.344**	2736.120***	42.941^{NS}	5.070^{NS}
Treatment (T)	1	170.253^{NS}	31.041^{NS}	.192**	7201.613***	697.687****	130.680****
Cow (C)	5	3352.190^{NS}	33.793^{NS}	.198**	6434.270***	18.513^{NS}	3.183
Residual (R)	4	8950.931	60.161	.025	185.037	11.844	2.455

[a] g/k OM disappeared in rumen.

[b] g/kg OM truly fermented in rumen.

^{NS} (P>.10)

** (P<.05)

*** (P<.01)

**** (P<.001)

(r=.65, P<.05), and ruminal K concentrations (r=.54, P<.07), but correlated negatively with ruminal digestion of OM (r=-.81, P<.01) and ruminal Na concentration (r=-.53, P<.07). Ruminal microbial N outflow estimated using adenine correlated only with water intake (r=.71, P<.01) and ruminal liquid outflow (r=.62, P<.03). Correlations of RMNO with kinetic measurements indicate that other factors may contribute to effects of high mineral treatment on RMNO.

Efficiencies of microbial N production (EMNPa and EMNPt) increased (P<.01) with high-mineral treatment (Table 16). There were no differences (P>.10) between estimates using cytosine and adenine. Values of EMNPa and EMNPt were in the range reported by Johnson (1982) for a variety of experimental conditions and according to values reported by Kang-Meznarich and Broderick (1980) for diets containing urea. These authors reported EMNPt of 24.8, 22.7 and 21.1 for nonlactating cows fed a diet based on ground corn, cottonseed hulls, mineral salts contents similar to our control treatment and 1.1, 1.6 and 2.3 percent of urea, respectively.

Both estimates of efficiencies of microbial N production, EMNPa and EMNPt, calculated using cytosine were correlated with ruminal digesta outflow, (r=.65, P<.02) and (r=.61, P<.04), respectively. In the case of adenine, only EMNPt correlated with ruminal digesta outflow (r=.59, P<.05). Different studies (Harrison et al., 1975; Isaacson et al.,

et al., 1975; Cole et al., 1976; Kennedy et al., 1976) indicated a positive effect of ruminal liquid outflow on efficiency of microbial N production. This effect has been related to a reduction in ruminal maintenance cost of bacteria (Isaacson et al., 1975; Harrison and McAllan, 1980), changes in microbial composition, and dilution of cell densities and microbial inhibitor substances (Bergen et al., 1982) as ruminal digesta outflow increases. In the present study, means of bacterial maintenance requirement calculated by the method of Pirt (1965) and using EMNPt were 6.45 and 4.25 g OM truly digested in the rumen/g-cell-h for control and high-mineral treatment, respectively. Also, it appears microbial cell chemical composition was modified by high-mineral treatment since microbial nucleotide-to-N ratios were changed (Table 16). However, it is not clear if this change in chemical composition was produced by a change in chemical structure of bacteria or for an ecological shift in bacterial population.

Variability of abomasal cytosine concentration over a 5-day sampling period (Table 19, 20) was not affected by period, treatment, cow and sampling day ($P>.10$). With respect to adenine (Table 19, 20) variability was affected by period ($P<.05$) and sampling day ($P<.10$). However, T*D and C*D interactions were significant ($P<.01$ and $P<.05$, respectively). Abomasal cytosine concentration was decreased by high mineral treatment, which was associated with higher outflow of ruminal digesta. The T*D interaction could be

TABLE 19. EFFECTS OF DIETARY TREATMENT, PERIOD, COW AND SAMPLING DAY ON ABOMASAL CONCENTRATION OF CYTOSINE AND ADENINE[a]

	Cytosine	Adenine
	------ mol/kg digesta -----	
Period		
A	34.2	41.8
B	34.5	51.8
Standard error	.8	1.0
Treatment		
Control	35.7	45.8
High-mineral	32.9	47.8
Standard error	.8	1.0
Cow		
1	36.8	41.5
2	34.6	46.7
3	34.0	49.6
4	35.3	53.9
5	31.8	43.0
6	33.5	45.9
Standard error	1.3	1.7
Day		
1	37.6	49.1
2	33.3	48.7
3	33.9	48.1
4	33.3	43.9
5	33.6	44.3
Standard error	1.2	1.6

[a]Means

TABLE 20. ANALYSIS OF VARIANCE FOR ABOMASAL CONCENTRATION OF CYTOSINE AND ADENINE

Source	d.f.	Mean Squares	
		Cytosine	Adenine
		--- μ/mol/kg digesta----	
Period (P)	1	1.38^{NS}	1506.00**
Treatment (T)	1	113.99^{NS}	59.20^{NS}
Cow (C)	5	28.94^{NS}	200.56^{NS}
P*T*C[a]	4	127.50***	168.72***
Day (D)	4	40.03^{NS}	76.85*
P*D	4	4.61^{NS}	49.32^{NS}
T*D	4	7.29^{NS}	134.50***
C*D	20	39.46*	75.69**
Residual	16	18.18	29.90

[a]Pooled interactions (P*T + P*C + T*C + P*T*C)

^{NS}(P>.10)

* (P<.10)

** (P<.05)

***(P<.01)

produced by periodical changes in ruminal digesta outflow and contribution of dietary adenine since as ruminal digesta outflow increases adenine escaping ruminal degradation may increase. The fact that abomasal concentration of cytosine was not affected by sampling day is important since number of samples may be reduced. However, it would appear necessary in individual experiments to define if sampling period during the day affects abomasal concentration of these bases.

Organic matter disappearing in the rumen (ROMD) was reduced ($P<.01$) by high-mineral treatment (Table 21, 22). A negative correlation was found for ROMD and ruminal digesta outflow ($r=-.66$, $P<.02$). This effect of high-mineral treatment on ROMD could be associated to a faster removal of OM from the rumen, which reduced microbial action on OM. Hogan and Weston (1967) found a negative effect of ruminal digesta turnover on ROMD. In the present experiment, ROMD also correlated negatively with ruminal K concentration ($r=-.69$, $P<.01$). Bennink et al. (1978) suggested that mineral salt supplementation may reduce ruminal microbial activity due to an increase in osmolality. However, using in situ bag technique, Rogers and Davis (1982b) did not obtain significant effect on DM disappearance and ruminal liquid outflow with intraruminal infusion of a constant volume of an aqueous solution containing 0, 200 and 600 g NaCl to steers fed a high roughage diet. In their

TABLE 21. DIETARY TREATMENT EFFECTS ON RUMINAL DIGESTION, DIGESTIBILITY COEFFICIENTS AND NITROGEN BALANCE[a]

Measurement	Treatment		
	Control	High-Mineral	S.E.
Organic matter disappearance in rumen (ROMD), %	51.6	37.7	1.2
Organic matter truly fermented in rumen (ROMF)[b], %	62.0	51.0	1.4
Apparent OM digestibility (ApOMD), %	52.2	50.4	1.2
Apparent N digestibility (ApND), %	56.9	53.5	1.1
Acid detergent fiber digestibility (ADFD), %	29.3	31.7	1.4
Nitrogen balance, g/day	20.0	-1.0	7.0

[a]Means.

[b]Using average of ruminal microbial N outflow estimated with both microbial markers.

TABLE 22. ANALYSIS OF VARIANCE FOR RUMINAL DIGESTION, DIGESTIBILITY COEFFICIENTS AND NITROGEN BALANCE

Source	d.f.	Mean Squares					
		OM disap. in rumen (ROMD)	OM truly fermented (ROMF)	AP. OM digestib. (ApOMD)	Ap. N digestib. (ApND)	ADF digestib. (ADFD)	N balance (NB)[a]
		%					
Period (P)	1	20.5^{NS}	.20^{NS}	.1^{NS}	.2^{NS}	26.4^{NS}	.0010^{NS}
Treatment (T)	1	578.2****	3.57***	9.5^{NS}	34.7*	16.8^{NS}	.0010^{NS}
Cow (C)	5	18.0^{NS}	.24^{NS}	13.2^{NS}	5.0^{NS}	50.3*	.0001^{NS}
Residual (R)	4	8.2	.13	8.1	7.3	12.5	.0002

[a]Degrees of freedom for residual = 3

^{NS}(P>.10)

* (P<.10)

***(P<.01)

****(P<.001)

experiment, ruminal osmotic pressure changed from 301 to 322 mOsm/kg of fluid, respectively. Bergen (1972) reported that cellulose digestion was not impaired until osmotic pressure was greater than 350 mOsm/kg of fluid.

Organic matter truly fermented in the rumen (ROMF) was greater than ROMD (Table 21, 22), since ROMF includes OM that was incorporated into microbial mass. High-mineral treatment also decreased ($P<.01$) ROMF but the magnitude of the effect was less than that on ROMD.

There was no significant treatment effect ($P>.10$) on apparent OM (ApOMD) and total ADF digestibility (ADFD) in the whole gastrointestinal tract as determined by total collection of feces (Table 21, 22). However, apparent N digestibility (ADFD) was decreased ($P<.10$) by high-mineral treatment (Table 21, 22). Different studies indicated little change in ApOMD, ApND and ADFD when ruminal liquid outflow was increased with dietary NaCl (Rogers et al., 1982) or $NaHCO_3$ (Rogers and Davis, 1982a). However, ApND decreased when ruminal liquid outflow increased by increasing feed intake or dietary roughage content (Bull et al., 1979). Similar to suggestions of others (Bull et al., 1979; Rogers and Davis, 1982a; Rogers et al., 1982), in the present study effects of ruminal kinetics on digestibility of dietary components may be confounded by specific effect of chemical or physico-chemical means used to change ruminal kinetics. Nitrogen balance (NB) was not affected ($P>.10$)

by high-mineral treatment (Table 21, 22). However, there was a trend ($P<.14$) to a lower NB with high-mineral treatment.

Neither milk yield and composition nor gross efficiencies of milk production were affected by high-mineral treatment (Table 23, 24). However, milk-fat content tended to increase with high mineral. Using similar dietary Na and K contents as in this study, Beede et al. (1983a) found increases in milk production between 3 and 5 percent with high-mineral diets. Rogers et al. (1982) reported no significant effect on milk yield and composition when altering ruminal liquid outflow by adding 2 percent NaCl to a basal diet.

TABLE 23. DIETARY TREATMENT EFFECTS ON MILK YIELD, COMPOSITION AND GROSS EFFICIENCY[a]

Measurement	Treatment		
	Control	High-Mineral	S.E.
Yield, kg/day	17.30	17.20	.30
Fat content, %	3.31	3.61	.13
Crude protein content, %	3.37	3.33	.17
Somatic cell count (x 1000)	8.00	3.00	3.00
Gross efficiency:			
Kg milk/kg DM intake	.78	.77	.67
Kg milk/kg CP intake	6.86	6.91	.88

[a]Least squares means.

TABLE 24. LEAST SQUARES ANALYSIS OF VARIANCE FOR MILK YIELD, COMPOSITION AND GROSS EFFICIENCY

	Yield		Fat		Crude Protein		Somatic Cell Count		Gross Efficiency: DM Intake		Gross Efficiency: CP Intake	
	d.f.	Mean Squares	d.f.	Mean Squares	d.f.	Mean Squares	d.f.	Mean Squares	d.f.	Mean Squares	d.f.	Mean Squares
	kg/day		%		%		(X 10^{-5})		kg/kg			
Period (P)	1	1.3801^{NS}	1	.0400^{NS}	1	.0003^{NS}	1	.0504^{NS}	1	3.3708^{NS}	1	.0800^{NS}
Treatment (T)	1	2.1709^{NS}	1	.4320^{NS}	1	.0083^{NS}	1	.0219^{NS}	1	2.8227^{NS}	1	1.7128^{NS}
Cow (C)	4	736.4272**	4	.3667^{NS}	4	.2475**	4	.0383^{NS}	5	3.0885^{NS}	5	4.7265^{NS}
P*T*C[a]	3	26.7110****	3	.2717^{NS}	3	.0106***	1	.0191^{NS}	-	---	-	---
Day (D)	6	9.9179***	1	.5780**	1	.0020^{NS}	1	.0011^{NS}	-	---	-	---
P*D	6	7.8556**	1	.0053^{NS}	1	.0003^{NS}	1	.0018^{NS}	-	---	-	---
T*D	6	.6836	1	.3413^{NS}	1	.0083**	1	.0080^{NS}	-	---	-	---
C*D	24	1.4592^{NS}	4	.0267^{NS}	4	.0020^{NS}	4	.0074	-	---	-	---
Residual	16	2.2305	3	.0606	3	.0606	3	.0057	4	2.7142	4	4.6976

[a]Pooled interactions (P*T + P*C + T*C + P*T*C)

^{NS}(P>.10)

**(P<.05)

***(P<.01)

****(P<.001)

CHAPTER 5

SUMMARY AND GENERAL DISCUSSION

In vitro and in vivo experiments were carried out to study effect of potassium (KCl) on apparent microbial urea utilization, and effect of dietary KCl and NaCl additions on ruminal kinetics and urea utilization by ruminal microorganisms. An enhancement of ruminal urea utilization may have practical implications since urea is less expensive than many natural protein sources.

The first in vitro batch-culture experiment was designed to study effect of potassium (KCl) on utilization of urea by ruminal microorganisms at different fermentation intervals. Treatments were in a 4x4x5x5 factorial arrangement. Concentrations of added potassium (K) and urea (U) were 0, .87, 1.74 and 2.62 g K/liter of fermentation fluid (0, 1.67, 3.35 and 5.04 g KCl) and 0, 53, 106 and 159 mg U/liter. Fermentation intervals were: 2, 4, 6, 8 and 10 h. Cultures consisted on .3 g of a semi-purified mixture (75 percent corn starch and 25 percent cellulose) and 20 ml of inoculum (1 ruminal fluid:4 buffer). Buffer used was a modification of McDougall's artificial saliva with 20 percent more buffering capacity and without potassium salt additions. Treatment effects were expressed as

apparent microbial nitrogen (N) utilization, which was estimated as N disappearance by difference between fermentation fluid N plus urea treatment N contribution at zero time minus final amount of NH_3-N in fermentation fluid at end of the fermentation time interval. Trial was replicated five different days, with one observation per treatment in each day. All main effects, including day of ruminal fluid collection (R) were significant as well as two and three-way interactions ($P<.001$), with exceptions of R*K*U and K*U*T. Independent of interactions, increasing K decreased AMNU but increasing U and T increased it. However, AMNU only decreased about 7 percent when K increased from 0 to 2.62 g/liter. Thus, a second in vitro experiment was designed with the same objectives and methodology but using greater concentrations of K and U.

In Experiment 2, treatments were in a 4x4x5x4 factorial arrangement. Concentrations of K and U were 0, 1.51, 3.02 and 4.53 g K/liter (0, 2.90, 5.80 and 8.73 g KCl) and 0, 143, 286 and 429 mg U/liter. Fermentation intervals were same as in Experiment 1. Trial was replicated four different days, with one observation per treatment on each day. All main effects and two and three-way interactions were significant ($P<.001$). Results were quite similar to those obtained in Experiment 1. Apparent microbial N utilization decreased as K increased. This effect of K on microbial response may be associated with an increase in osmolality.

Independent of interactions, U and T increased AMNU. Similar to Experiment 1, extent of AMNU per unit of K decreased as U increased, and extent of AMNU per unit of U decreased as K increased. In both experiments, a partial microbial adaptation to K seemed to occur after 6 h fermentation intervals. Study indicated that addition of potassium, as chloride salt, to a medium containing about .25 g K/liter decreased apparent microbial utilization of urea at all K concentrations tested.

An in vivo experiment was designed to study effects of changing ruminal kinetics with dietary sodium and potassium chloride on microbial protein synthesis using urea as sole source of supplemental nitrogen. Other ruminal measurements, digestibility and lactational performance were measured also. Two dietary treatments were studied using a simple reversal design. Dietary concentrations of Na and K were .19 and 1.03, and .58 and 1.86 percent (dry basis) for control and high-mineral treatments, respectively. Basal diet (dry basis) consisted of 55.6 percent ground corn, 40 percent cottonseed hulls, 1.5 percent urea and 2.9 percent other minerals and vitamins. Six midlactation Holstein cows fitted with ruminal and abomasal cannulae were fed ad libitum and milked twice daily in individual tie-stalls. Cows were fed with a pre-adaptation diet (15 days) containing the same cocentration of urea but intermediate concentrations of Na and K compared to the

experimental treatments. Each 10-day sampling period was preceded by a 10-day adaptation period. Ytterbium-marked fecal fiber, CrEDTA, lignin and cytosine and adenine were used as markers of solid phase, liquid phase, digesta and microbial protein mass, respectively.

There was no treatment effect ($P>.10$) on DM intake, ruminal dilution rate (D) and solid turnover rate (STR), but high-mineral treatment increased water intake ($P<.001$), ruminal volume ($P<.01$), liquid outflow ($P<.05$) and digesta outflow. Least squares means for ruminal liquid outflow were 180.9 and 207.5 liter/day, and for ruminal digesta outflow were 170.5 and 248.8 kg/day with control and high-mineral treatment, respectively. High-mineral treatment increased ruminal osmolality ($P<.05$) and K concentrations ($P<.001$), decreased Na concentrations ($P<.01$), but did not affect pH or ammonia concentrations ($P>.10$). Ruminal VFA concentrations were not affected ($P>.10$), but molar proportion of acetate was increased ($P<.01$) and propionate was decreased ($P<.05$) by high-mineral treatment.

Ruminal microbial N outflow ($P<.01$) and efficiencies of microbial N production ($P<.001$) were increased by high-mineral treatment. Both estimates were not different ($P>.10$) between microbial markers. Average ruminal microbial N outflows were 246.4 and 314.6 g/day, and efficiencies of microbial N production were 22.4 and 39.8 g/kg OM disappearing in rumen, and 18.6 and 29.4 g/kg OM truly fermented in rumen with control and high-mineral treatments, respectively.

Variability of abomasal cytosine concentration was not affected by period, treatment, cow and sampling day ($P>.10$). In the case of adenine, variability was affected by period ($P<.05$) and sampling day ($P<.10$), but not by treatment and cow ($P>.10$).

Organic matter disappearing in the rumen ($P<.001$) and apparent N digestibility ($P<.10$) were decreased by high-mineral treatment, but there was no effect ($P>.10$) on apparent OM digestibility, ADF digestibility, and N balance. There was no effect ($P>.10$) on lactational performance.

The study indicated that high-mineral treatment (.56 percent Na and 1.86 percent K) increased some measurements of ruminal kinetics and amount and efficiency of microbial N production. Also, it demonstrated that augmenting ruminal kinetics increased utilization of urea by microorganisms.

Readily available energy is one of limiting factors in ruminal utilization of urea. With dietary crude protein contents higher than 12 percent, utilization of urea or other NPN sources requires higher supplies of energy that are possible under many economic situations. In lactating cows modification of ruminal kinetics may increase utilization of urea without additional supply of energy since part of the effect on ruminal kinetics is to increase efficiency of microbial nitrogen production by reduction in the energetic cost associated with ruminal microbial maintenance.

This may have considerable impact since urea is less expensive than most of natural protein sources.

Studies from Florida (Dairy Research Unit, University of Florida) indicate higher requirements of Na and K for lactating cows than those recommended by NRC (1978), especially during heat stress. Under different dietary conditions of the present work, recommended Na and K concentrations (.6 percent Na and 1.8 percent K) as chloride salts increased ruminal kinetics. This may facilitate substitution of natural protein sources by urea or other NPN sources since little additional costs will be necessary for manipulating ruminal kinetics.

Also, in tropical countries where mixtures of molasses-urea are used commonly for meat and milk production, an improvement in ruminal utilization of urea would be important. In these production systems, molasses may be provided as source of energy to the animal and also for improving microbial urea utilization due to a more readily available supply of energy. However, there is evidence indicating that molasses reduces ruminal kinetics, which may increase ruminal energy loss and may decrease urea utilization. Thus, an increase in ruminal kinetics may have tremendous potential in these animal production systems. Manipulating ruminal kinetics with dietary salts such as sodium and (or) potassium may be economically feasible in

these countries. However, a better understanding of these factors in the whole animal will be required.

Results of the present research have contributed to the understanding of effects of higher amounts of potassium and sodium chloride on ruminal kinetics and microbial utilization of urea. Also, these results demonstrated that an increase in ruminal liquid or digesta outflow improved microbial urea utilization, resulting in an increase of 30 and 80 percent in microbial nitrogen outflow and efficiency of microbial nitrogen utilization, respectively. However, it will be necessary to obtain additional understanding of relationships among different dietary urea contents and ruminal kinetics in order to make adequate recommendations regarding utilization urea under different production conditions.

GLOSSARY

ADFD = acid detergent fiber digestibility, %

ADR = microbial adenine-to-N ratio, μmol/g

AMNC = abomasal microbial nitrogen concentration, g/kg wet digesta

AMNU = apparent microbial nitrogen utilization, mg/100 ml

ApND = apparent nitrogen digestibility, %

ApOMD = apparent organic matter digestibility, %

CT = control treatment

CYR = microbial cytosine-to-N ratio, μmol/g

D = ruminal dilution rate, %/h

DM = dry matter

EMNPa = apparent efficiency of microbial nitrogen production, g/kg OM disappearing within rumen

EMNPa-ad = apparent efficiency of microbial nitrogen production estimated using adenine, g/kg OM disappearing within rumen

EMNPa-cy = apparent efficiency of microbial nitrogen production estimated using cytosine, g/kg OM disappearing within rumen

EMNPt = true efficiency of microbial nitrogen production, g/kg OM truly fermented within rumen

EMNPt-ad = true efficiency of microbial nitrogen production estimated using adenine, g/kg OM disappearing within rumen

EMNPt-cy = true efficiency of microbial nitrogen production estimated using cytosine, g/kg OM disappearing within rumen

HMT = high-mineral treatment

K = added potassium, g K^+/liter

LCD = lignin content of abomasal digesta, kg/kg wet material

LI = lignin intake, kg/day

NB = nitrogen balance, g/day

NH_3-N = nitrogen as ammonia, mg/100 ml

NPN = non-protein nitrogen

OM = organic matter

OMI = organic matter intake, kg/day

R = day of ruminal fluid collection

RDF = ruminal digesta outflow, kg wet material/day

RK = ruminal potassium, g/liter

RLF = ruminal liquid outflow, liter/day

RMND = apparent organic matter disappearing from rumen, %

RMNO = ruminal microbial nitrogen outflow, g/day

RMNOad = ruminal microbial nitrogen outflow estimated using adenine, g/day

RMNOcy = ruminal microbial nitrogen outflow estimated using cytosine, g/day

RNa	= ruminal sodium concentration, g/liter
ROMF	= true organic matter fermented in rumen, %
RV	= ruminal volume, liter
SRT	= ruminal solids turnover rate, %/h
STR	= ruminal solids retention time, h
T	= fermentation interval, h
U	= added urea, mg/liter
VFA	= volatile fatty acid
WIN	= water intake, liter/day
Y(ATP)	= microbial yield/mol ATP produced in rumen

A P P E N D I X

TABLE 25. GROSS CORRELATION COEFFICIENTS (IN VIVO EXPERIMENT)

	WIN	ROS	RNa	RK	RV	RLF	RDF	RMNOcy	RMNOad	EMNPa-cy	EMNPa-ad	EMNPt-cy	EMNPt-ad	ROMD
WIN	1.00[a] .00[b]	.12 .69	-.68 .01	.46 .13	.53 .07	.61 .03	.73 .01	.77 .01	.71 .01	.43 .17	.55 .07	.43 .16	.54 .07	-.47 .12
ROS	.12 .70	1.00 .00	-.33 .30	.71 .01	-.15 .65	-.14 .67	.21 .51	.36 .25	.25 .44	.54 .07	.63 .03	.56 .06	.65 .02	-.42 .17
RNa	-.68 .01	-.32 .30	1.00 .00	-.84 .01	-.83 .01	-.42 .18	-.88 .01	-.53 .07	-.25 .43	-.58 .05	-.50 .09	-.57 .05	-.53 .08	.58 .05
RK	.46 .13	.70 .01	-.83 .01	1.00 .00	.57 .05	.13 .69	.77 .01	.54 .07	.14 .66	.74 .01	.66 .02	.75 .01	.68 .01	-.69 .01
RV	.53 .08	-.15 .65	-.83 .01	.58 .05	1.00 .00	.38 .21	.81 .01	.33 .29	-.01 .97	.37 .24	.18 .57	.19 .54	.19 .54	-.43 .16
RLF	.60 .04	-.14 .67	-.42 .18	.13 .69	.38 .22	1.00 .00	.49 .11	.49 .10	.62 .03	.14 .66	.20 .54	.14 .66	.21 .51	-.25 .42
RDF	.73 .01	.21 .51	-.87 .01	.77 .01	.81 .01	.49 .11	1.00 .00	.65 .62	.23 .47	.60 .04	.46 .13	.61 .04	.48 .11	.66 .02
RMNOcy	.77 .01	.36 .24	-.53 .07	.54 .07	.33 .29	.49 .10	.65 .02	1.00 .00	.58 .05	.79 .01	.76 .01	.81 .01	.75 .01	-.81 .01
RMNOad	.71 .01	.25 .44	-.25 .43	.14 .66	-.01 .96	.61 .03	.23 .47	.58 .05	1.00 .00	.18 .58	.57 .05	.19 .56	.56 .06	-.22 .48
EMNPa-cy	.43 .16	.54 .07	-.58 .05	.74 .01	.37 .23	.14 .66	.60 .04	.79 .01	.18 .59	1.00 .00	.85 .01	.99 .01	.86 .01	-.96 .01
EMNPa-ad	.54 .07	.62 .03	-.50 .09	.66 .02	.18 .57	.20 .54	.46 .13	.76 .01	.57 .05	.85 .01	1.00 .00	.84 .01	.99 .01	-.82 .01
EMNPt-cy	.43 .15	.56 .06	-.57 .05	.75 .01	.35 .26	.14 .66	.61 .04	.81 .01	.18 .58	.99 .01	.84 .01	1.00 .00	.85 .01	.96 .01
EMNPt-ad	.54 .06	.65 .02	-.53 .08	.68 .01	.20 .54	.21 .51	.48 .11	.75 .01	.56 .06	.86 .01	.99 .01	.85 .01	1.00 .00	.82 .01
ROMD	-.47 .12	-.42 .17	.58 .05	-.69 .01	-.43 .16	-.25 .43	-.66 .02	-.81 .01	.22 .48	-.96 .01	-.82 .01	-.96 .01	-.82 .01	1.00 .00

[a]Correlation coefficient.

[b]Probability <|R|

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BIOGRAPHICAL SKETCH

Manuel Humberto Ruiloba was born in Los Santos, Panamá, on February 15, 1948.

In 1971, he received his Bachelor's degree in Chemistry from the University of Panamá, Panamá. In 1973, he received his Master of Science degree from the Nutrition Institute of Central America and Panamá (INCAP), Guatemala, with a major in food science.

In 1974, he joined the Ministry of Agriculture, Panamá, and later in 1975 the Agricultural Research Institute of Panamá, as researcher in animal science.

In 1981, he enrolled in Graduate School at the University of Florida to pursue his studies toward the Doctor of Philosophy degree.

He is currently a member of the Latin American Society of Animal Science, the American Society of Animal Science, the American Society of Dairy Science, and the Panamanian Society of Animal Production.

He is married to the former Elizabeth S. De Freitas and has two children, Humberto Ariel and Elaine Itzel.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Harold H. Van Horn, Chairman
Professor of Dairy Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

David K. Beede, Co-chairman
Assistant Professor of Dairy Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Joseph H. Conrad
Professor of Animal Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Gordon M. Prine
Professor of Agronomy

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Charles J. Wilcox

Charles J. Wilcox
Professor of Dairy Science

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December 1984

Jack L. Fry

Dean, College of Agriculture

Dean for Graduate Studies and Research